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Title THE BONE STRUCTURE OF SOME MARINE VERTEBRATES

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This paper deals with the bone structure of albacore, fin whale and deep-sea rat-tail. The major components of bone - ash, organic, fat and water content - are expressed on a volumetric basis. It has been found that both rat-tail and whale bone tissue are highly mineralized and the mineralization occurs at the expense of water. The whale and albacore tissue densities were 1.856 grams/cm^3 and 1.557 grams/cm^3 respectively. Bone organ densities of whale, albacore and rat-tail were 1.161 grams/cm^3, 1.423 grams/cm^3 and 1.378 grams/cm^3, respectively. The differences in bone structure with respect to physiological and environmental factors are also discussed.

THE BONE STRUCTURE
OF SOME MARINE VERTEBRATES

by

SARGUN ALI TONT

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APPROVED:

Redacted for privacy

Professor of Oceanography, Department of Oceanography
In Charge of Major

Redacted for privacy

Chairman, Department of Oceanography

Redacted for privacy

Chairman of School Graduate Committee

Redacted for privacy

Dean of Graduate School

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Dedicated to Dr. James Arnold, Providence Hospital, Portland, Oregon, teacher and friend who, more than anybody else, is responsible for the completion of this thesis.

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THE BONE STRUCTURE OF SOME MARINE VERTEBRATES

INTRODUCTION

The purpose of this paper is to determine the bone density and the volume of the major components of the bone - ash, water, organic content + carbon dioxide, and fat - of the following marine vertebrates:

1. Albacore tuna, *Thunnus alalunga* (Bonnaterre),
2. Deep-sea rat-tail of grenadier, *Coryphaenoides* sp.,
3. Fin whale, *Balaenoptera physalus* (L.).

Relatively little is known about the bone structure of marine vertebrates, and, to my knowledge, this is the first attempt to analyze the major components of bone of the above marine vertebrates on a volumetric basis. The majority of the published work deals either with morphology or chemical analysis of the bone and bone ash rather than the density and the major components of the bone (e.g., 8, p.455-466, 9, p.1037-1038, 7, p.753-766, 6, p.97-98).

An important concept of this paper is the expression of the composition of bone on a volumetric basis. Deakins (3, p.429-435) showed the usefulness of this method when studying water content of teeth enamel, and additional contributions also came from Robinson and Elliott (11, p.167-168), Arnold (1, p.167-175), and Arnold and Tont (2). Basing the results of bone analysis on a weight basis is often misleading because the organic, inorganic, fat and water content all have different densities. On a percentage basis an increase of inorganic material having a high density results in an apparent decrease of organic matter even if its volume is unchanged (3, p.429). In this paper, the organic content + carbon dioxide, water, ash and fat contents are all expressed as grams per unit volume of the bone.

Some distinctions must be made regarding two types of bone materials: the bone tissue itself without fat or medullary tissue, and the whole bone organ including fat and medullary interstitial material found in the spaces between the bone tissue. Further clarification is also necessary regarding the water content of the bone. There are two types of water in the bone: one in the interstices or holes which is loosely held, and the second type which is molecularly bound in the bone tissue (2).

MATERIALS AND METHODS

The bone specimens used in this study were taken from the vertebral column of an albacore and a rat-tail and from a section of a whale rib. All three vertebrates were caught off the Oregon coast. Bone samples were frozen until volume determinations were made, approximately 72 hours later. Bone samples consisted of 11 albacore vertebrae, approximately 0.8 cm in length and 0.9 cm in diameter; 12 rat-tail vertebrae, each 0.2-6.3 cm in diameter; and 28 whale rib sections taken from the same rib, each ranging from 1.5-2.0 cm x 2.0-2.3 cm x 2.4-5.4 cm.

Since the bone as an organ has a different density than bone as a tissue, two types of density measurements, each requiring different procedures, were carried out.

The following procedure for measuring the density of the bone tissue, developed by Arnold and Tont (2), was used to determine the densities of the above samples. Bones were thoroughly washed in a jetstream of water, thus removing either all or part of the fat and connective tissue. Fat not removed by washing was later removed by soxhlet extraction, and the original volumes were corrected. Next the cleaned bone samples were placed in separate beakers filled with cold, distilled water which were placed in a desiccator. Using a mechanical pump, the pressure inside the desiccator was reduced to 4 mm of mercury, the boiling point of water, for 40 minutes, thus enabling the captured gas in the samples to escape. No additional bubbles were observed at the end of the 40 minutes. The samples were then weighed in the water while suspended from a thin copper wire.

In order to calculate volume and density, it was necessary to weigh the bones after removal of excess water (that water which is not in the bone tissue itself but on the surface and in the channels of the bone). In the case of whale rib and albacore vertebrae, this was done by centrifugation at 8 x 10^3 rpm, about 10,000 g's, for one-half hour at 0°C.

Since the rat-tail vertebrae were so small that the bone tissue could not be separated from surrounding material, the tissue density was not determined directly. Nevertheless, a good approximation was made on the basis of the ratio between the organic, inorganic and fat-free dry weights (see Results and Discussion sections).

The bone organ densities of the albacore and rat-tail were determined in the same manner as tissue densities except washing and centrifugation were omitted. This enables the bone to retain its loose interstitial water and fat content. After weighing in water, the samples were blotted on a piece of filter paper and reweighed. Since the distilled water was at 4°C, fat loss was negligible. In the case of the bone organ density of the whale rib, large rectangular samples were cut, and volume was determined directly by measuring the three sides of the rectangle with calipers.

Water content of the bone tissue, or the difference between post-centifugation wet weight and dry weight, was obtained by drying the bone samples for 72 hours at 100°C, and then in a desiccator evacuated to one mm of mercury and using P_2O_5 as a desiccant. After three hours of cooling in the evacuated desiccator, the bones were weighed. The above procedure enables one to remove the chemically bound water of the bone.

Fat extraction was accomplished by drying the bones at 100°C and collecting the fat on a piece of filter paper. Later both the bone and the filter paper were placed in a soxhlet apparatus and extracted in an ether alcohol mixture for 24 hours. After the extraction the solutions were put in pre-weighed beakers and solvent evaporated at room temperature in a stream of air. After the evaporation, the fat residue was weighed. The Physics and Chemistry Handbook value of .927 grams/cm^3 was used for fat density.

To determine ash and organic content the specimens, whose hydrated densities were previously determined, were ashed at 530°C for 48 hours in a muffle furnace (5, p.6). Melting pellets were used to calibrate the pyrometer of the muffle furnace. As the muffle cooled, samples were removed at 300°C and placed in a P_2O_5 desiccator and allowed to cool _in vacuo_ prior to weighing. Special attention was given to loss of bone through chipping. When chipping occurred, the small pieces of bone were weighed with the individual bones to which they belonged. Robinson (12), points out that in addition to oxidizing and driving off the organic material during the ashing of bone a variable amount of chloride, potassium, intercrystalline water, and most important of all carbon dioxide is lost. These account for an error of 4% in ash weight (1, p.169).

The individual ash densities were calculated using Archimedes' principle. Bone ash was degassed in water, weighed, dried at 100°C, and weighed again.

RESULTS

The results of this study are summarized in Tables 1-6. Table 1 gives the average hydrated tissue densities and the major components of the bone of the three vertebrates on a gram per unit volume basis. Table 2 gives the major components on a percentage-of-volume basis, i.e., volume of the major component divided by volume of the bone tissue. Tables 3-5 deal with bone organ densities. Here the volume of the total bone organ is given, not the volume of the tissue alone. H_2O/cc denotes the total water content (molecularly bound water + loose water).

The whale rib has a high tissue density. On a percentage-of-volume basis, its ash and organic content + carbon dioxide components occupy roughly equal volumes (Table 2). We also note that both the ash and water content of the whale bone tissue are linearly related to hydrated density (Figs. 1 and 2). The small standard deviation of organic content + carbon dioxide suggests that it has a more or less constant volume (Table 1).

Whale bone, as an organ, has a very low density due to high fat and water content (Table 3).

Albacore has a low bone tissue density. More than half of the bone is made of water (Table 2). On a percentage-of-volume basis its

Table 1. Densities and major components of bone tissue (grams/cc).

	Density (grams/cm^3)	S.D.	H_2O/cc	S.D.	Ash/cc	S.D.	Organic +CO_2/cc	S.D.	Dry Density	S.D.
Whale	1.856	.021	.321	.045	.977	.020	.547	.006	2.285	.008
Albacore	1.557	.025	.542	.029	.608	.067	.400	.015	2.203	.073
Human	1.910	.025	.2050	.020	1.060	.020	.645	.002	2.250	.030

	H_2O/cc	Ash	Organic + CO_2
Whale	32	31	37
Albacore	54	19	27
Human	27	34	29

Table 2. Major bone tissue components on percentage-of-volume basis.

Organism	Density	S.D.	H_2O/cc	S.D.	Ash/cc	S.D.
Whale	1.161	.022	.396	.024	.329	.018
Albacore	1.423	.015	.430	.035	.479	.051
Rat-tail	1.378	.024	.727	.019	.446	.028

Table 3. Densities and major components of bone organ (grams/cc).

Organism	Fat-Free Dry-Weight/cc	S.D.	Fat/cc	S.D.	Organic +CO_2/cc	S.D.
Whale	.513	.076	.252	.014	.184	.010
Albacore	.800	.082	.193	.019	.321	.044
Rat-tail	.651	.041	.000	.000	.207	.018

Table 4. Major components of bone organ (grams/cc).

Organism	Ash	H_2O	Organic + CO_2	Fat
Whale	11	40	22	27
Albacore	15	43	21	21
Rat-tail	14	73	13	00

Table 5. Major bone organ components on percentage-of-volume basis.

Organism	Ash Density	Organic + CO_2 Density
Whale	3.122	1.506
Albacore	3.168	1.505
Rat-tail	3.165	1.556
Human	3.150	1.522

Table 6. Major bone component densities (grams/cm^3).

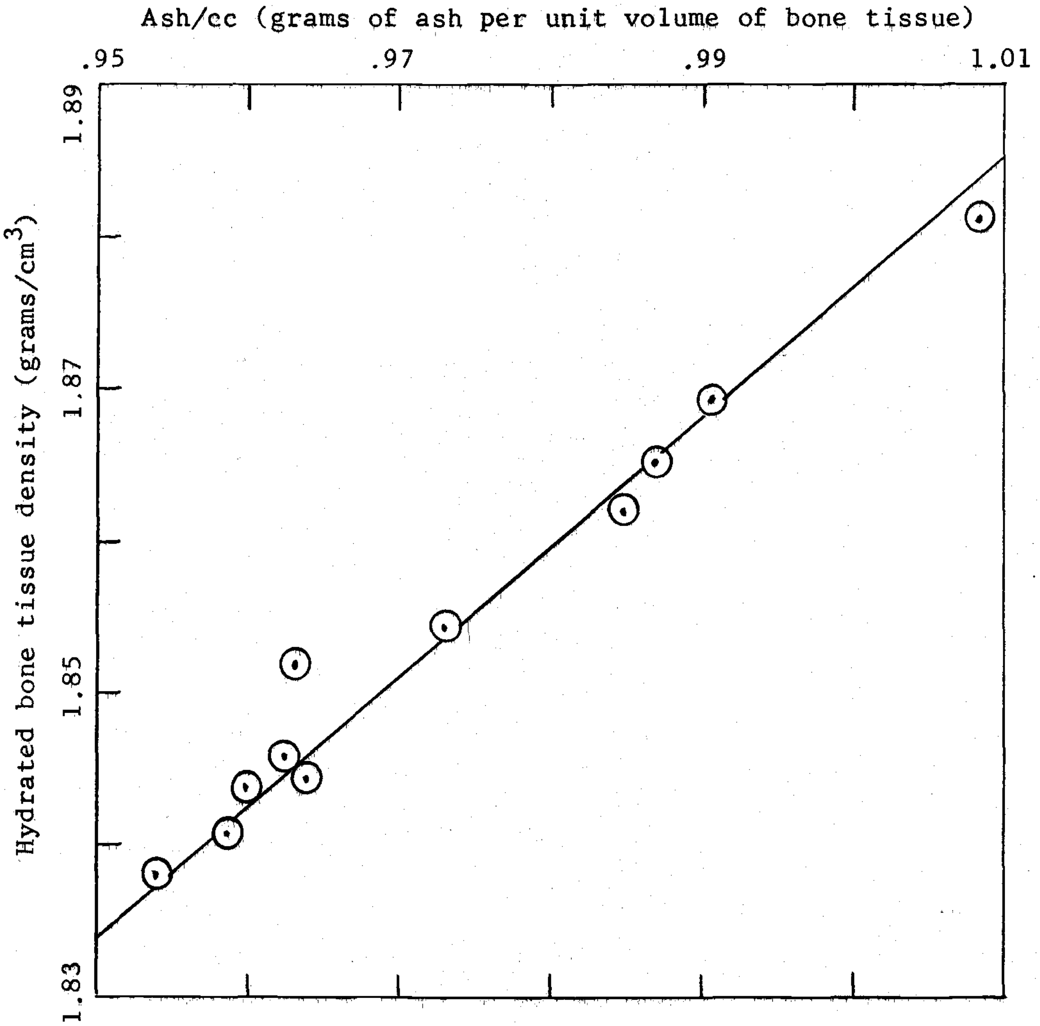

Figure 1. Bone tissue density versus ash/cc of fin whale.

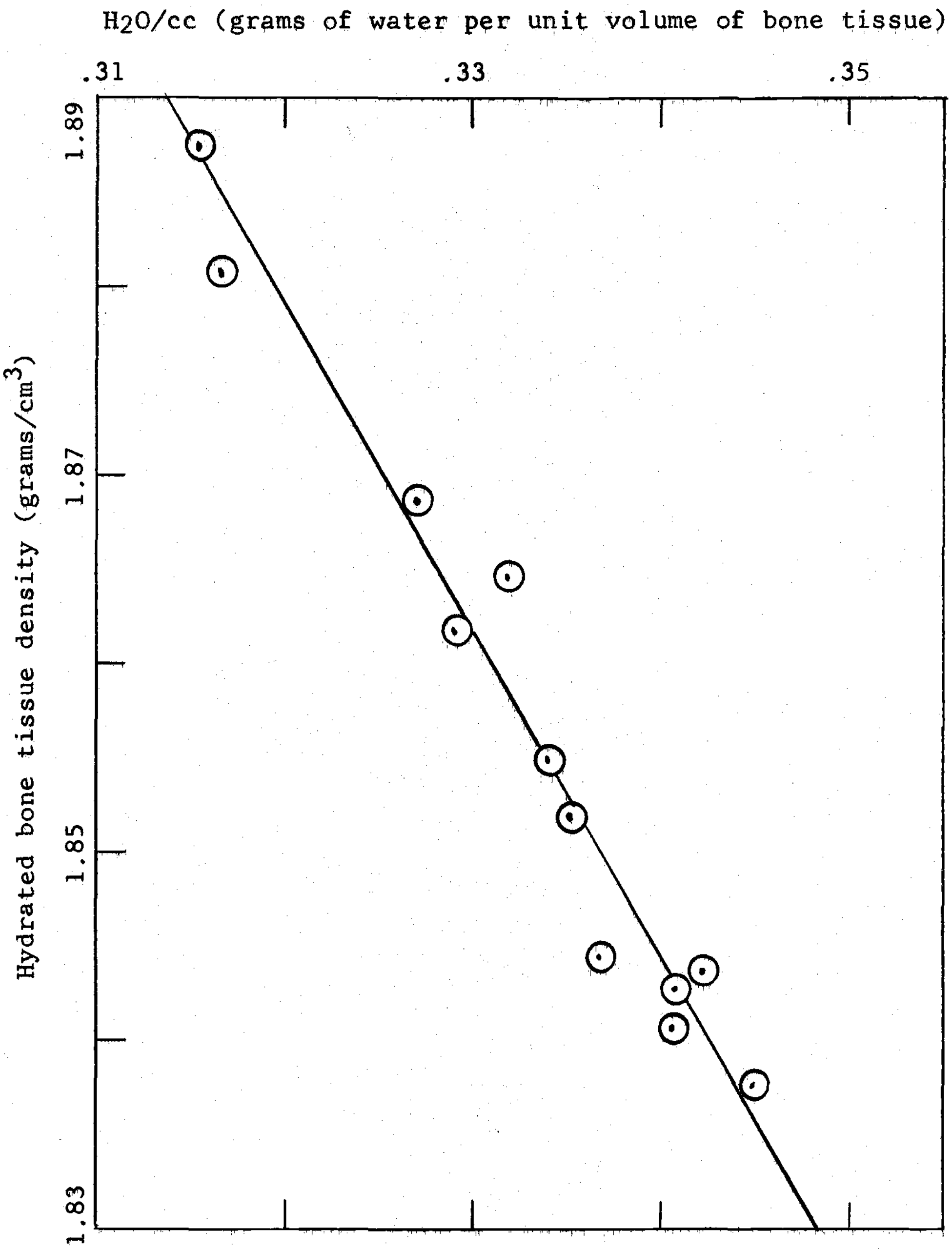

Figure 2. Bone tissue density versus H_2O/cc of fin whale.

organic content + carbon dioxide component is much higher than its ash component. There is a linear relationship between ash, water and density in albacore (Figs. 3 and 4).

Albacore has a relatively high bone organ density. Its bone organ is highly mineralized with respect to its organic content + carbon dioxide component.

A bone tissue density was not obtained directly for rat-tail because of difficulty in separating the bone tissue from adjoining material.

The rat-tail vertebra as an organ has a very high water content - almost 73% of its volume was water. On an organ basis, it is mineralized bone with little organic content + carbon dioxide.

We also found that the component densities of the three marine vertebrates and that of man show a very close relationship (Table 6).

DISCUSSION

Bone as a Tissue

The large variation in the bone tissue densities of the three marine vertebrates may be related to phylogenetic, structural, and physiological differences.

When we compare the bone tissue of a whale with the bone tissue of another mammal, man (Fig. 8), we note a strong resemblance. Both bones are spongy or highly trabecularized. Because of this similarity in structure one would expect both bone samples to have similar ash, organic + carbon dioxide and water content. If we examine Table 1, we note that indeed that is the case, whale having a slightly lower ash and organic content + carbon dioxide and a slightly higher water concentration than man. Since these proportions are similar, the overall hydrated tissue densities of whale and man are very similar. On further examination of Table 1, one would think that whale tissue bone is ideally constructed for a strong, but elastic skeleton. This point becomes clear if we think of the bone tissue as connective tissue whose intertwisted strands of fiber contain both a very elastic (organic) and rigid (ash) material in high quantities (Fig. 5). Since whale is a large, fast-swimming mammal (13, p.112), it needs this strong skeleton to permit the tremendous accelerations and propulsive forces experienced during swimming.

Albacore's vertebra tissue is markedly different from that of man and whale - it is a more compact bone with relatively fewer holes (Fig. 6). The difference between the ash, organic content + carbon dioxide, and water content of the albacore bone as compared to whale bone can be explained on the basis of bone morphology. According to Moss (9, p.1037-1038), teleost fish bone has a different cellular structure than a mammalian bone. Both ash and water content of the bone sample of the same organisms have a linear relationship with density (Figs. 1, 2, 3, and 4). This suggests that mineralization in marine vertebrate bone tissue occurs at the expense of water. Organic matter + carbon dioxide being more or less constant,

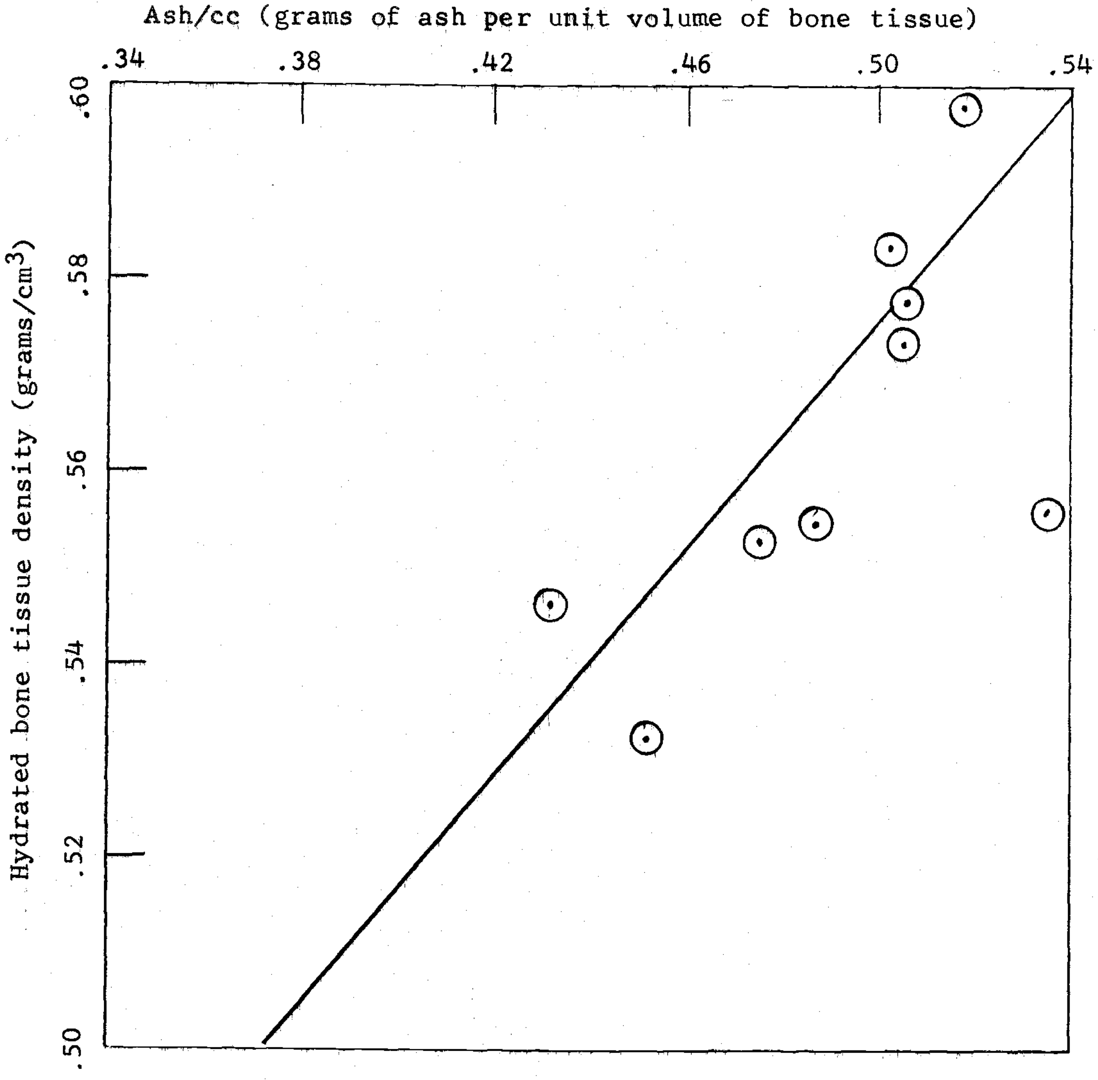

Figure 3. Ash/cc versus bone tissue density of albacore.

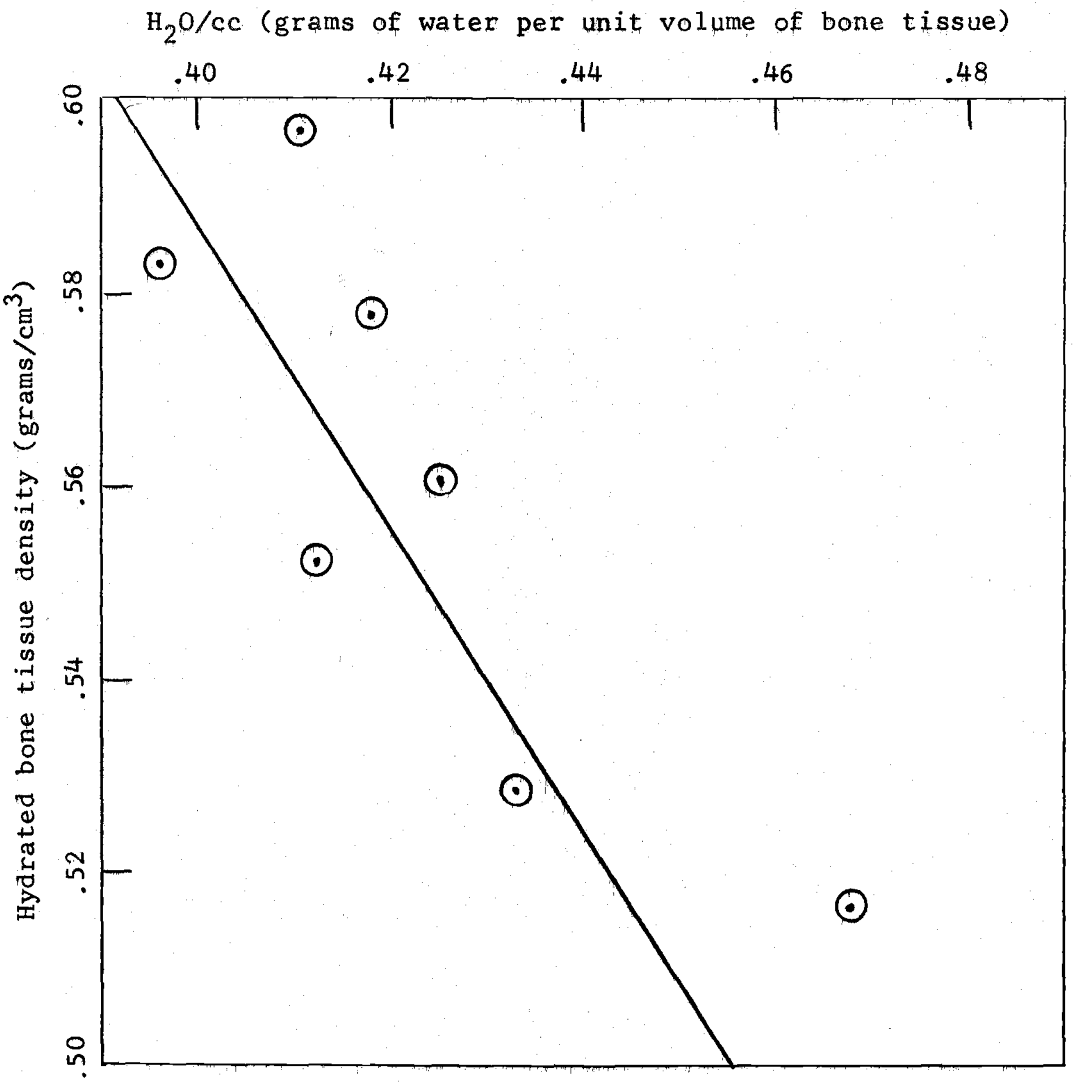

Figure 4. H_2O/cc versus bone tissue density of albacore.

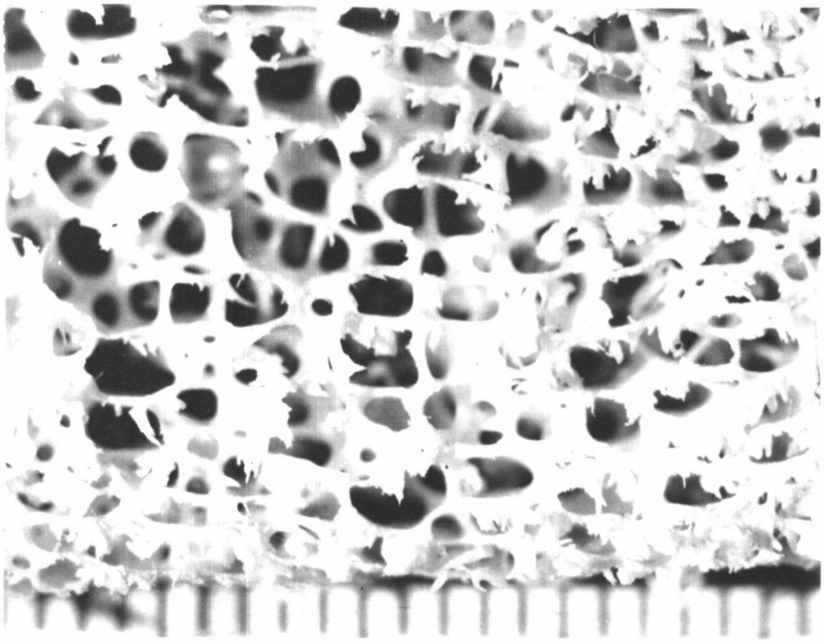

Figure 5. Photograph of whale rib section showing haversian canals (Scale in mm's).

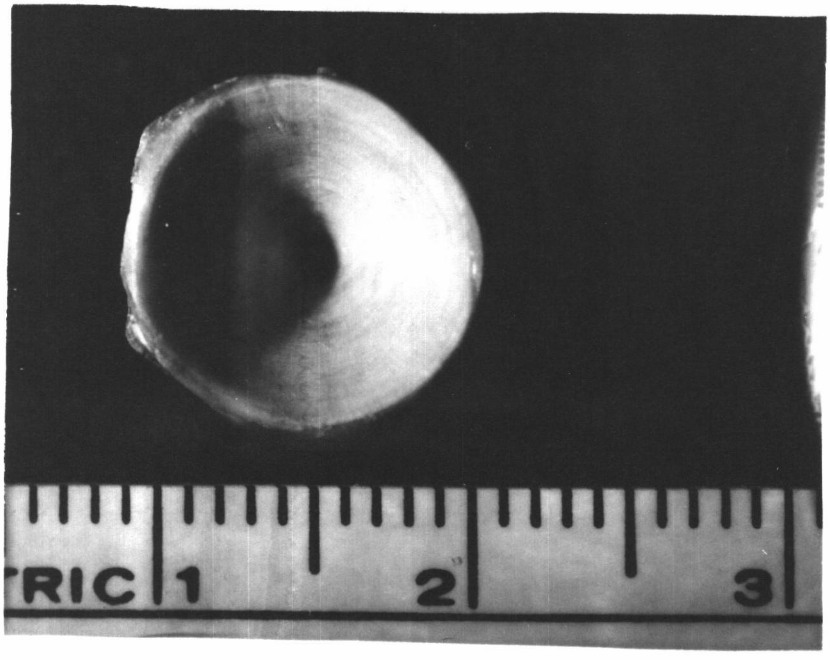

Figure 6. Photograph of centrum of albacore vertebra (Scale in mm's).

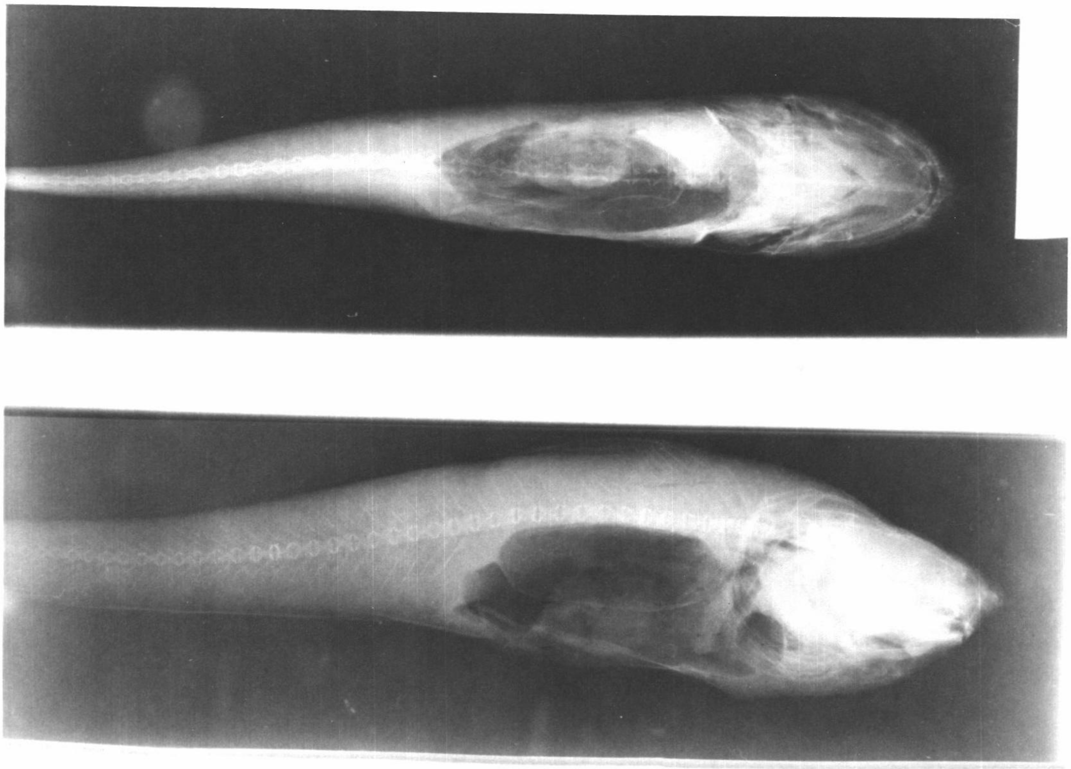

Figure 7. Radiograph of rat-tail.

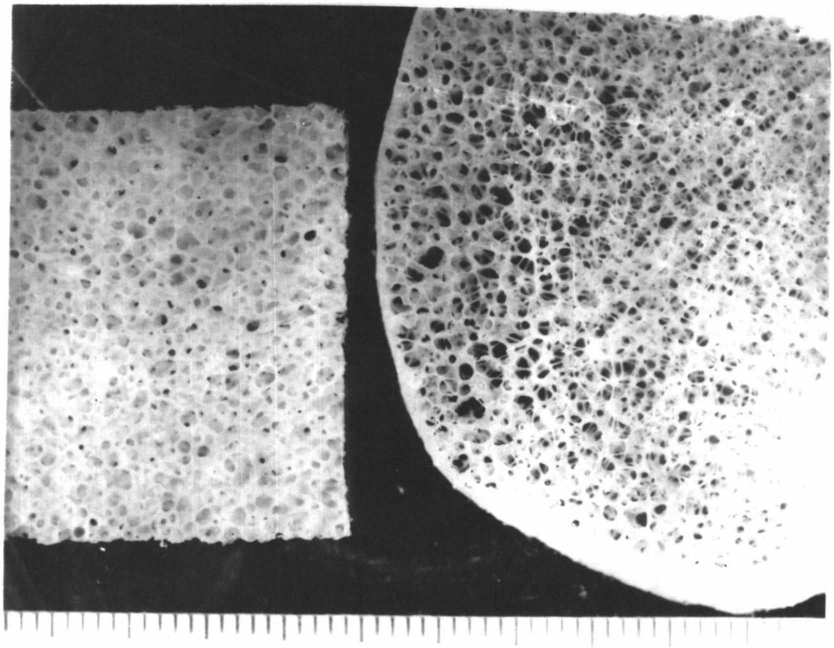

Figure 8. Photograph of whale bone (left) and human bone (right) (Scale in mm's).

This very important point was proven earlier by several researchers using terrestrial mammalian bone (3, p.429-435, 11, p.167-168, 2).

As it was mentioned earlier, we were not able to determine the tissue density of rat-tail, but we can make a reasonable approximation. Rat-tail bone tissue is very highly mineralized on a dry weight or organic content + carbon dioxide basis (Table 4). Since the densities and weights of the ash and organic components are known we can determine their ratios on a volumetric basis. Then, let us make an assumption that rat-tail bone tissue has the same organic + carbon dioxide concentration as the albacore. This may sound like a weak assumption, but we know from a survey of bone literature that related organisms have similar organic mater matter + carbon dioxide concentration (5, p.14). Now, using the above-mentioned volume ratios, and assuming that rat-tail has a similar organic matter + carbon dioxide concentration as the albacore, we find that on a percentage-of-volume basis rat-tail will have an ash concentration value of 28% compared to the albacore's ash concentration of 19% and whale's 31%. This suggests that the rat-tail bone tissue is very highly mineralized and only slightly lower than that of whale or man.

One interesting point here is, that according to some researchers (7, p.756, 6, p.98), most deep-sea fishes that do not have a gas-filled swimbladder have a weakly ossified skeleton as compared to some coastal fishes with gas-filled swimbladders. As the rat-tail has a swimbladder, and is presumably neutrally buoyant, therefore a well-mineralized skeleton does not create any buoyancy problems (Fig. 9).

Bone as an Organ

In the first part of this discussion we have noted the strong resemblance between the bone tissue of both whale and man. But any similarity is practically nonexistent as far as organ densities are concenred. In whale bone as an organ, I noted a very high concentration of fat and an absence of any medullary tissue (blood vessels, etc.). This high concentration of fat is obviously important for buoyancy purposes. According to Slijper (13, p.109), 14% of a whale's body weight is skeleton, which itself is about one-half fat. If it wasn't for the high content of fat in the holes of the bone it would be difficult for a whale to have neutral buoyancy. The absence of medullary tissue suggests that there is little hemapoietic activity in the rib sections.

Albacore, on the other hand, has a higher bone organ density than the whale in spite of a very high water and fat content. Therefore, this high bone organ density decreases its buoyancy. Although albacore, whose bone tissue is less mineralized than the whale bone, it has more bone tissue per unit volume of the whole bone organ than the whale (Fig. 10). Therefore, it also has a strong axial skeleton which would be needed by a fast, continually-swimming fish.

If we examine the individual densities of the major bone components of the three marine vertebrates and that of man, we note a

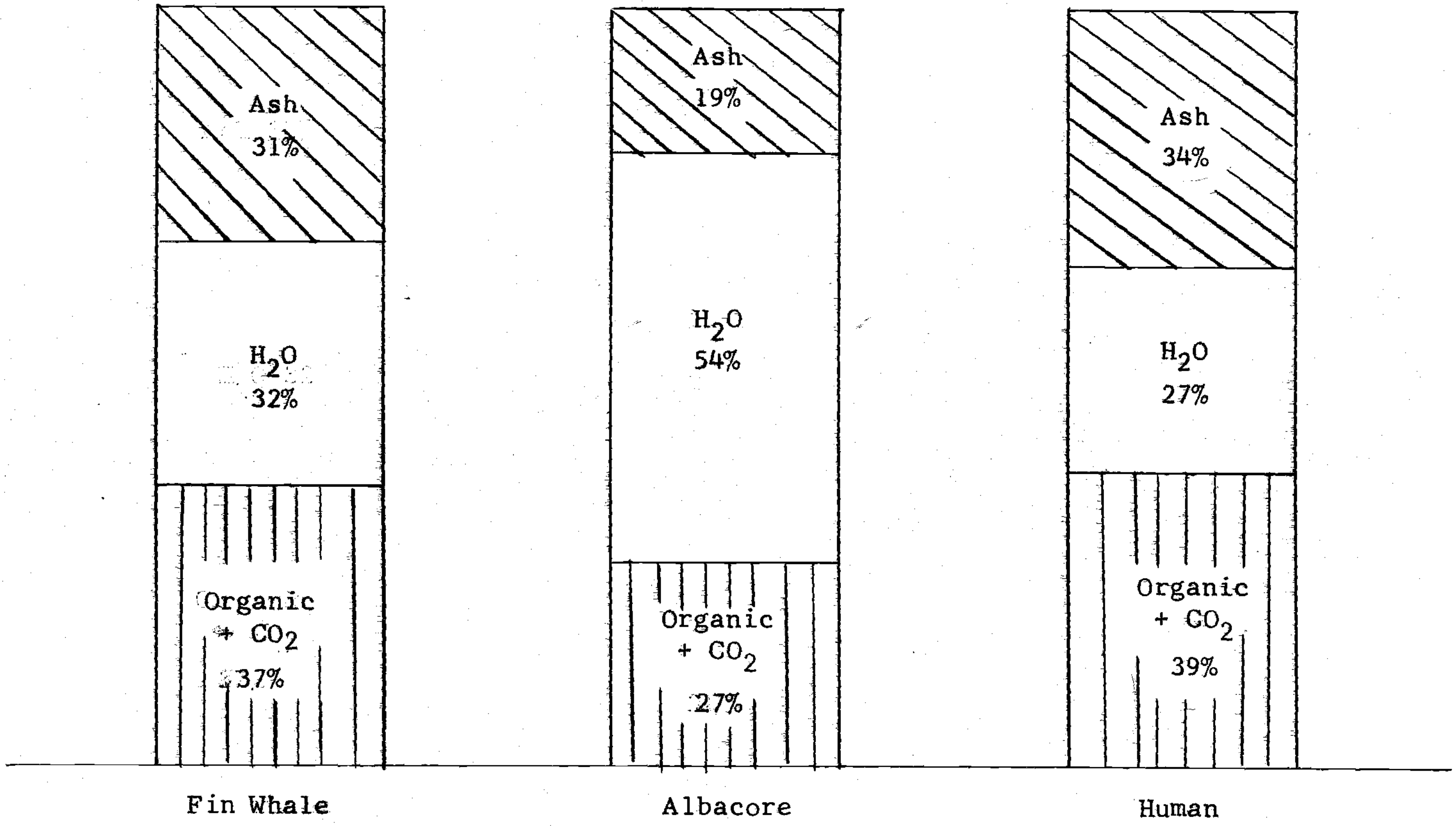

Figure 9. Major components of bone tissue on a volumetric basis.

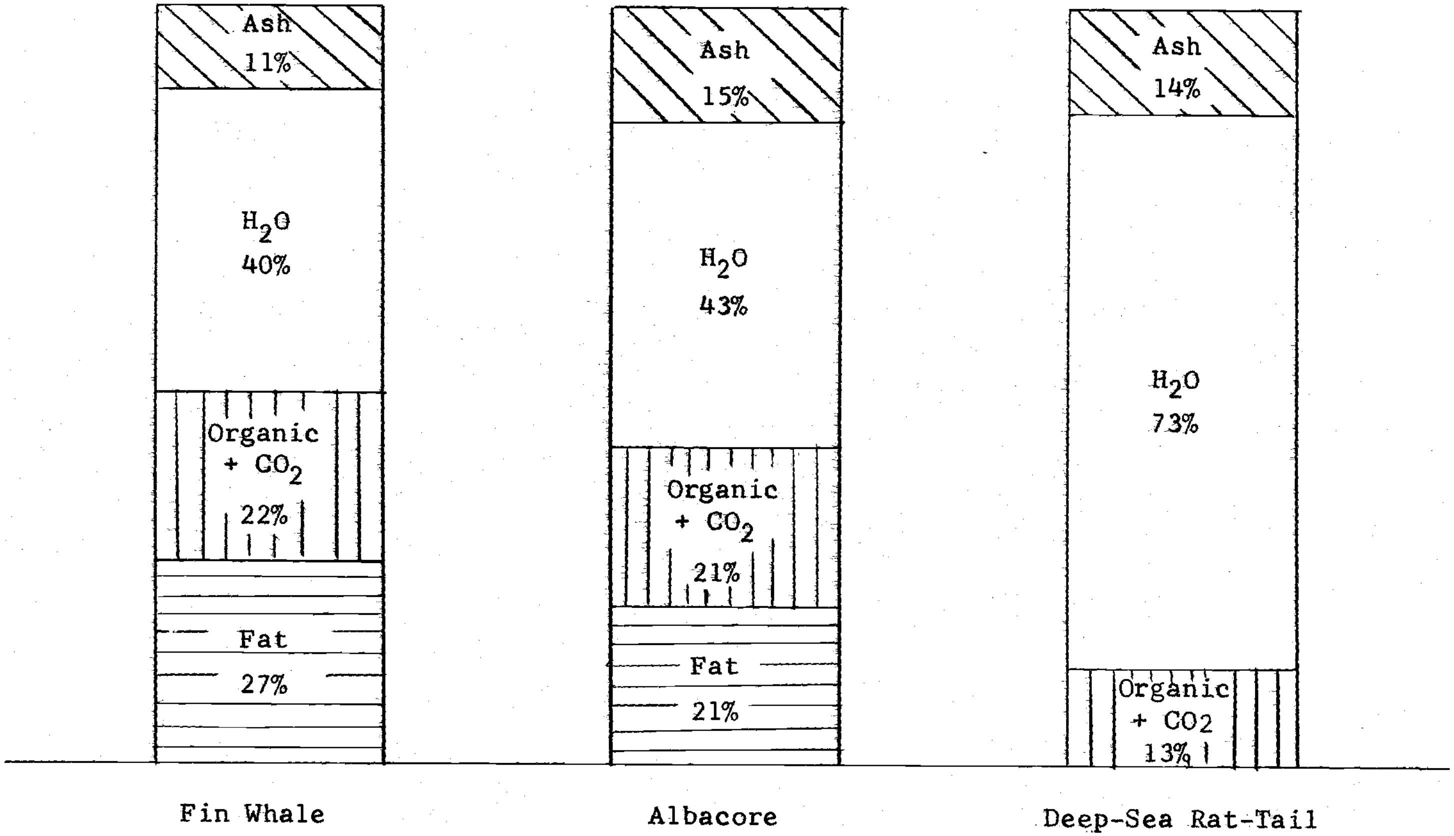

Figure 10. Major components of bone organ on a volumetric basis.

strong resemblance (Table 6). Although chemical analyses of each component were not made, this similarity suggests the possibility that the basic elements of these components are of the same nature.

When the average of ash/cc and water/cc is plotted versus hydrated density values of man, whale and albacore, a very interesting linear relationship is obtained (Figs. 11 and 12). This relationship between such diverse vertebrates with widely different densities suggests a general feature of bone structure that density is directly related to ash content and inversely to water content.

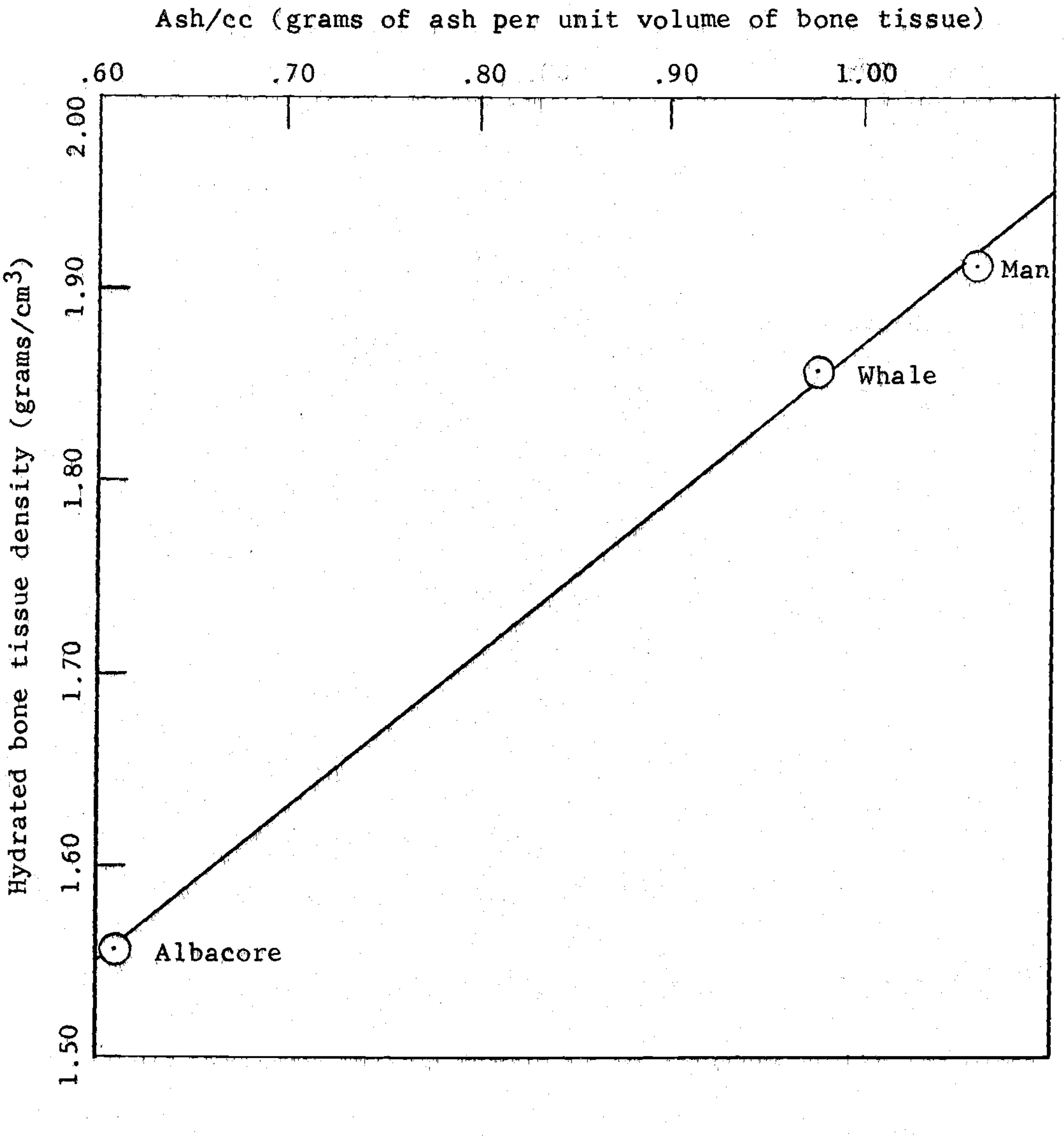

Figure 11. Ash/cc versus hydrated bone tissue density of whale, albacore and man.

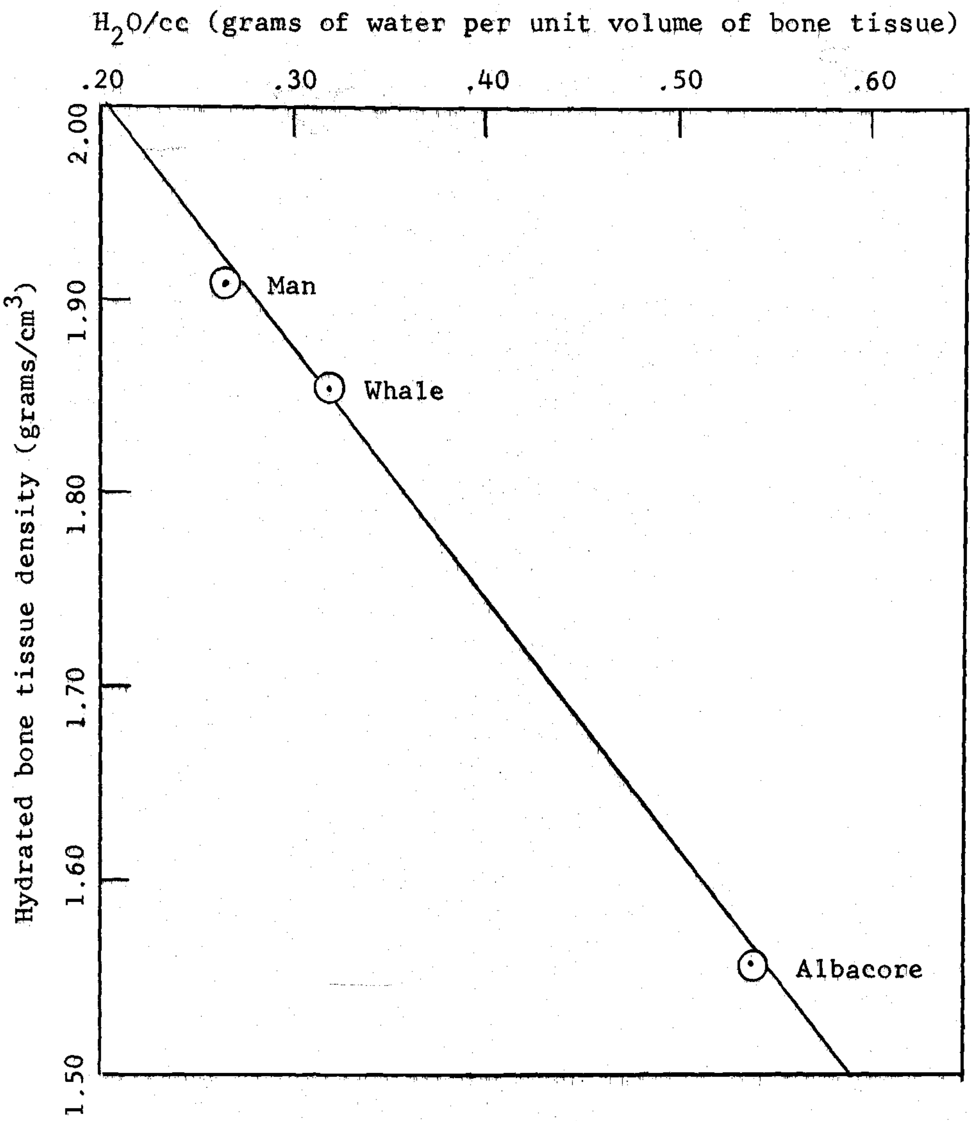

Figure 12. H_2O/cc versus hydrated bone tissue density of whale, albacore and man.

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