



ADIPOCYTE AREA AS INDICATORS OF ENERGY RESERVE OF GRAY WHALE *Eschrichtius robustus*, IN SAN IGNACIO LAGOON, BAJA CALIFORNIA SUR, MEXICO



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INTRODUCTION

Body condition (BC) is an indicator of an individual's and population's physical condition, upon which reproductive success and general health depend on¹. Photo-identification, photogrammetry, and total lipids analysis have been conducted to evaluate BC on the gray whale^{2,3}. However, its fat layer's degree of energy deficiency is still poorly known. This study evaluates the area of adipocytes as a reference measure of BC. This evaluation will be carried out on gray whales that visit Laguna San Ignacio in the winter season.

MATERIAL AND METHODS

San Ignacio Lagoon is located on the Pacific coast of Baja California Sur, Mexico (Fig. 1). Calves, mothers and single individuals (whales without calves) from different BC categories can be found here. So, biopsies of all three types were taken. The superficial fat layer was recovered and fixed in 10% formalin. Simultaneously, photographs of the biopsied specimens were taken, and they were classified according to a BC scale: Normal, Acceptable or Poor (Fig. 2).

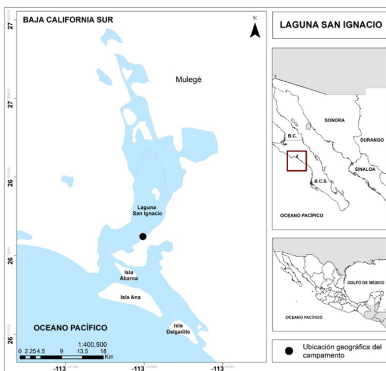


Figure 1. Location of the Laguna de San Ignacio.

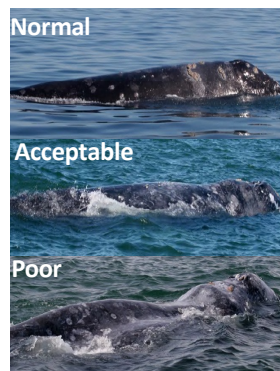


Figure 2. Body condition categories.

Photomicrographs with the 10x objective were taken in two fields adjacent to the basal lamina, i.e., dermis and hypodermis (Fig. 3a). According to Castrillón *et al.*, (2017)⁴, the cell area was recorded only in complete adipocytes, i.e., cells with a well-defined circumference by a collagen membrane (Fig. 3b).

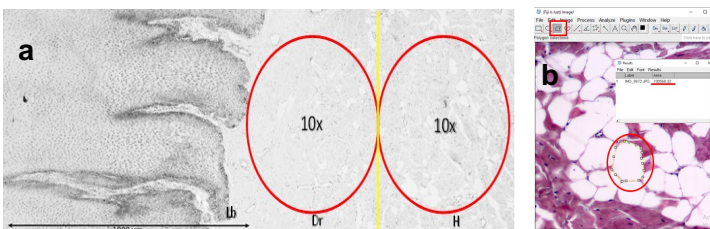


Figure 3. a) Gray whale skin. Lb, basal lamina; Dr, dermis; H, hypodermis. The red circle exemplifies the range of the 10x objective. b) Complete adipocytes, the red circle indicates how the measurements were made.

RESULTS

117 biopsies representing mothers (22), calves (31), and single individuals (64) were analyzed. Single individuals presented normal (14), acceptable (26) and poor (23) BC.

In some cases, two biopsies per individual were taken, 5 and up to 50 days apart. In most biopsies of adult individuals, a decrease in the area of adipocytes was recorded (Fig 4). In contrast, in calf samples an increase in adipocyte area was recorded (Fig. 5) (Table 1).

Table 1. Difference in adipocyte area (AA μm^2) between the 1st and 2nd biopsies.

Individual	Day between 1 ^a and 2 ^a biopsy	AA μm^2 1 ^a biopsy	AA μm^2 2 ^a biopsy
Single	5	44,069	31,628
Mother	39	32,608	54,541
Mother	20	39,581	35,481
Mother	11	45,145	40,355
Calf	44	17,836	35,252
Calf	50	13,812	12,593
Calf	39	6,739	23,262
Calf	20	15,832	32,811
Calf	11	27,630	30,667
Calf	2	29,020	30,897

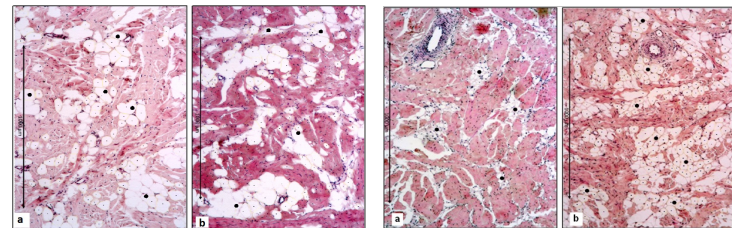


Figure 4. Adipocytes (black dots) in a single individual gray whale. a) First biopsy, March/15/2020 and b) second biopsy, March/20/2020. Photographs of preparations stained with H-E, 10x.

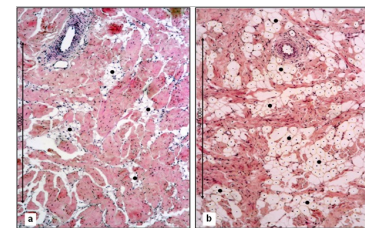


Figure 5. Adipocytes (black dots) from a gray whale calf. a) First biopsy, January/29/2020 and b) Second biopsy, March/08/2020. Photographs of preparations stained with H-E, 10x.

Calf adipocytes increased their area significantly during the winter stay (from 6,739 μm^2 to 44,792 μm^2), and mother adipocytes increased only slightly (from 31,577 μm^2 to 43,247 μm^2) (Fig. 6).

In contrast, no differences were detected across the adipocyte area among the categories established *a priori* in single individuals (normal = 39, 098 \pm 7, 298 μm^2 ; acceptable = 42, 451 \pm 10, 881 μm^2 ; poor = 37, 052 \pm 13, 583 μm^2) (Fig. 7).

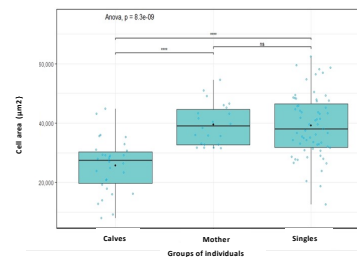


Figure 6. ANOVA of AA of the types of gray whale groups.

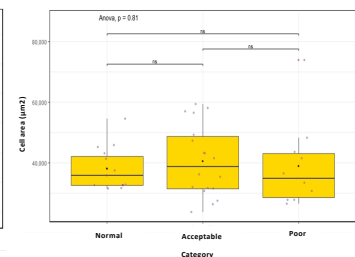


Figure 7. ANOVA of AA of the different categories of gray whale body condition.

DISCUSSION AND CONCLUSION

The analysis of the area of adipocytes applied in the study is a valuable tool for evaluating the state of the energy reserves of gray whale calves. However, its application to adult adipocytes appears to be limited.

The slight variation observed in both mothers and adults of different gray whale BC can be associated with the characteristics of the fat of the superficial layer, the layer from which the samples for the present study were obtained. In other studies, also carried out in the surface layer, no differences were recorded in the size, shape, several adipocytes^{5,6} and lipid richness⁷. The stability of the superficial fat is associated with functions of thermoregulation and thermal insulation⁸ and is even considered physiologically inert or stable⁹.

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