Rufford Small Grant for Nature Conservation

Final Report

The manatees of the Panama Canal Watershed

Presented by:

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PROJECT SUMMARY

The purpose of this study was to evaluate the distribution and genetic composition of the manatee population in Lake Gatun in the Panama Canal Watershed. Believed to be re-introduced into the lake in 1964, the manatees of Lake Gatun have not been studied since the late 1980's. Three different methodologies were used in this study: 1) interviews with local people, 2) aerial and boat surveys, and 3) genetic analysis using mitochondrial DNA.

A total of 44 interviews revealed 59 manatee sightings on the lake. In addition, the interviews revealed a previously unknown source of information: the captain's logs of the Panama Canal Aquatic Vegetation Control Division. These logs recorded a total of 17 manatee deaths in the lake between 1995 and 2007. The cause of death is not clear, but in most cases it appears to be collisions with boats. Aerial and boat surveys revealed a maximum of 16 manatee sightings, including several females with calves. This is much higher than expected based prior research.

Samples for genetic analysis were difficult to find in the lake. Only three tissue samples and two bone samples were acquired. Sampling of the only other known resident population in Bocas del Toro, resulted in 99 fecal samples collected for genetic analysis. Using mitochondrial DNA (mtDNA) analysis the results revealed that all three tissue samples from Lake Gatun and 64 viable samples from Bocas del Toro were the same haplotype, Haplotype J. These results indicate that there is no genetic variability in either population. However, since mtDNA does not allow the identification of individuals, we recommend further studies be carried out using microsatellite markers in order to corroborate these results.

An adult and juvenile manatee (Trichechus manatus) during aerial surveys of Lake Gatun

The present study represents the first systematic study of this unique manatee population. Results have shown that manatees in the lake are more abundant than previously thought, and that they tend to congregate in specific areas of the lake. However, more studies are needed in order to determine factors affecting distribution.

This study also represents the first instance of genetic analysis of manatees in Panama, and the first time manatee feces have been successfully used to obtain DNA. The information obtained through molecular analysis highlights the dangers of carrying out relocation programs without proper ecological and genetic studies, particularly on such an endangered marine mammal. Further studies are needed to evaluate the true genetic health and conservation status of this population.

INTRODUCTION

The West-Indian manatee *Trichechus manatus* is considered Vulnerable by IUCN due mainly to habitat destruction and illegal hunting throughout most of its distribution (Lefebvre, 2001; IUCN, 2007) from Florida in U.S.A., Central America to northern Brazilian coast, including the Antilles.

In Panama information on manatees is scarce and the last study carried out on this species was in the late 1980's (Mou-Sue *et al*., 1990). There are two resident manatee populations in the country, one in Bocas del Toro, on the Northern Caribbean Coast, and the other in Lake Gatun, in the Panama Canal Watershed (Mou-Sue *et al*., 1990; Lefebvre, 2001) (Fig. 1). This second manatee population is believed to have been reintroduced in 1964 by the former Panama Canal Commission as part of an aquatic vegetation control program (MacLaren, 1967). They introduced one Amazonian manatee *T. inunguis* from Peru and nine West-Indian manatees *T. manatus* from Bocas del Toro in Lake Gatun to help control several species of aquatic plants. However, a few years later, some of the manatees escaped during a storm, and shortly after the remaining manatees were released, and the program was abandoned (MacLaren, 1967).

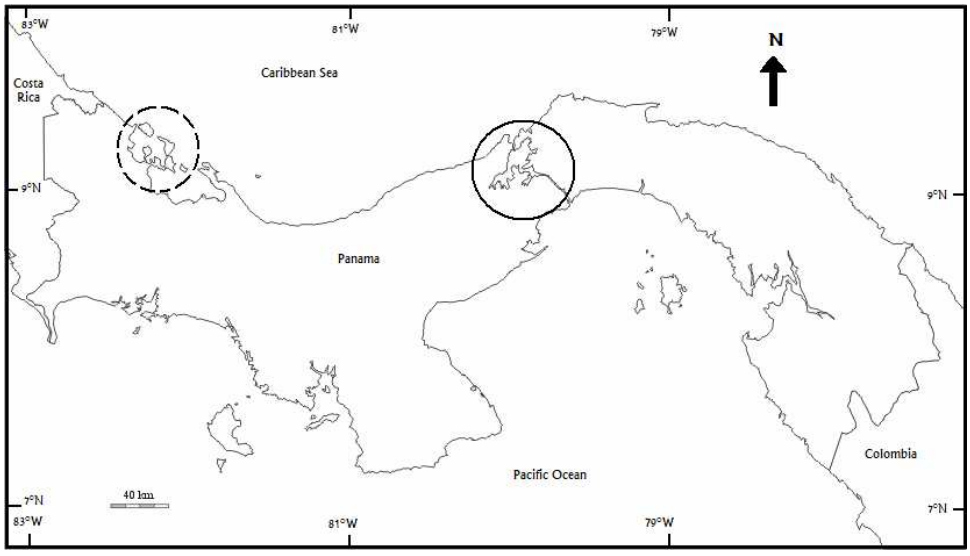


Fig.1 Location of Panama's resident manatee populations; one in Bocas del Toro (*dash- line circle*) and one in Lake Gatun (*solid line circle*).

It is not widely known that manatees existed in the Chagres River prior to its impoundment for the creation of Lake Gatun and the Panama Canal in 1904, but it is unclear whether or not these manatees survived the construction period of the Canal (Mou-Sue *et al*., 1990; MacLaren, 1967). Schad *et al*. (1980) estimated that there were approximately 25 manatees in the lake based on the proportion of males and females introduced in the 1960's; they also gathered over 50 manatee sightings through interviews with local people. A few years later, Mou-Sue *et al*. (1990) carried out aerial surveys in both Bocas del Toro and Lake Gatun. But after 5.5 hours of aerial surveys on the lake they sighted only one manatee. To date, there is no detailed systematic study of this unique manatee population to evaluate of the current status and what threats there are to their conservation. The purpose of this study was to assess the distribution and genetics of the manatees of the Panama Canal Watershed.

DESCRIPTION OF STUDY AREA

Lake Gatun is located in central Panama covering some 430km^2 (Fig. 2). It is an artificial freshwater lake created in 1906 when the Chagres River was impounded to build the Panama Canal. The tropical climate of the country provides an average annual temperature of 28°C, with an average annual rainfall of more than 2,000ml. The temperature of the lake oscillates around 28°C (25 – 31°C), and it has an average depth of 13m, though certain parts can reach a depth of 29m. Some 20 smaller rivers empty into the lake, and there are many small islands and coves along the lake edge providing a variety of habitats for manatees and other aquatic and terrestrial fauna. The typical vegetation in the lake includes *Eichhornia crassipes*, *Pistia stratiotes*, *Pontederia rotundifolia* and *Hydrilla verticillata*, all of which are part of the manatee's diet (Jimenez-Perez, 2000; TLBG *et al*., 2002).

FIG. 2 Map of principal study area: Lake Gatun. All ships that transit the Panama Canal go through this lake.

MAJOR ACTIVITIES AND RESULTS

To simplify the analysis of data the lake was divided into six sectors: I) Gaillard Cut South II) Gaillard Cut North III) Chagres River, IV) Barro Colorado, V) Northeast and VI) West (Fig. 3).

Personnel from the Gamboa Rainforest Resort during interviews. Interviewees were provided with a questionnaire and a map of the lake so they could mark areas in which they had sighted manatees.

A. Interviews with local people

The main goals of the interviews were to determine recent and historical sightings, most common causes of death, potential threats to manatee conservation, and to inquire about local people's perception of the manatees in the lake. In order to maximize manatee reports, interviews were centered on key members of public and private institutions that operate in and around Lake Gatun.

A total of 44 interviews were conducted between March and June 2007, which yielded 59 manatee sightings, 63% (37) corresponded to recent manatee sightings, and 22% (13) were historical sightings, including one sighting in 1977 and one in 1972. The sightings concentrated on the Chagres River (15%), both sections of Gaillard Cut (sectors I and II) (41%), Barro Colorado, Sector IV (24%) and Northeast, Sector V (14%) sectors. Only 21% (9) of respondents knew of the existing legislation prohibiting hunting of manatees, 78% (6) of which corresponded to the STRI game wardens and one member of the Ecological Police. In addition, 21% (9) of the respondents recalled seeing females with calves, but there was no specific time of the year when calves were seen more often. Finally, 64% (27) of respondents have seen at least one dead manatee in the lake. When questioned as to the cause of death, 26% (7) said they were the result of detonations during the widening and dredging activities in Gaillard Cut, while 37% (10) were attributed to collisions with boats.

Revision of documents

The captain's logs of the Aquatic Vegetation Control Unit of the Panama Canal Authority serve as a registry of the activities carried out by the unit's personnel for each day they are out on the lake. These activities include the removal of deceased animals; mostly alligators (*Crocodylus acutus*) and manatees.

The revision of captain's logs revealed many registries of manatee deaths in Lake Gatun which were previously unknown.

A total of 32 Captain's logs were revised, in which there were a reported 17 manatee deaths from 1995 to 2007. The greatest number of deaths was registered in 2007, when 4 deaths recorded. No deaths were recorded during 1998 or 2003. However, since the Aquatic Vegetation Control Unit only patrols a specific portion of the lake, not all manatee deaths are reported here. Also, quick decomposition and consumption by scavengers decreases the probability of manatee

carcass recovery. It is interesting that the highest number of deaths, thirteen deaths (76%), were registered in Gaillard Cut (Fig. 3).

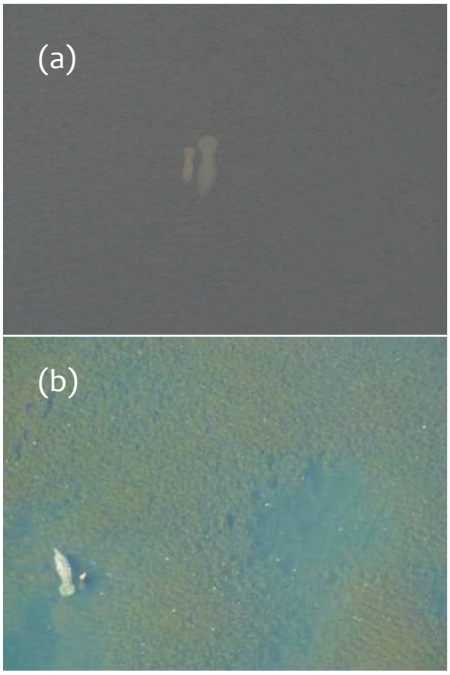
B. Boat and Aerial Surveys

Surveys were carried out in a 3m long fiberglass boat with a 25hp outboard engine, on five different occasions from January to June 2007, in areas where interviews mentioned possible manatee sightings. The boat used the outboard motor to get to and from these areas. Once at the survey sites the engine was turned off, and the area was surveyed using oars to minimize disturbance to any manatees or other wildlife in the immediate area.

Jorge Pascal (pilot), Giselle Muschett, Karla Aparicio and Isis Tejada moments before take-off during 3rd aerial survey of Lake Gatun, Oct. 2008

Aerial surveys were flown in a Robinson R44 helicopter, flying at an average altitude of 150m and speed of 180 km/h. The surveys were flown in 400m wide transects where manatee sightings were most probable (Hodgson *et al*., 2007). During each of the flights every time a manatee was spotted from the air each observer recorded the number of individuals seen, if they were in a group and the presence of calves (individuals of approximately less than 2m in close proximity to or accompanying a larger individual) (Morales-Vela *et al*., 2000).

Four boat surveys were conducted between March and June 2007. Each survey lasted approximately 4 hours. Two manatees were seen; one in the Chagres River (Sector III) and one in the Barro Colorado sector (Sector V) (Fig. 3). Aerial surveys were conducted in the dry season on 10 January and 14 January 2008 for a total of 2.25h, and a third aerial survey was conducted during the wet season on 8 October 2008 for 1.5h. A total of 12 manatee sightings were made during the surveys; 10 adults (83%) and two calves (16%) in the dry season. In the wet season survey a total of 16 manatees were sighted, 12 adults (75%) and 4 calves (25%), in spite of bad weather conditions and murky waters. This count was by far the largest number of manatees ever recorded in the lake.



(a) A manatee and calf sighted during 3rd aerial survey in October 2008. (b) A manatee sighted during 1st aerial survey in January 2007. Note the differences in water clarity.

In addition, certain biotic and abiotic factors appeared to influence the presence of manatees. During boat and aerial surveys, 92% of manatees were sighted near to forested and/or protected areas and areas with low (92%) to medium (8%) boat traffic. No manatees were sighted near agricultural lands that bordered the lake (West sector).

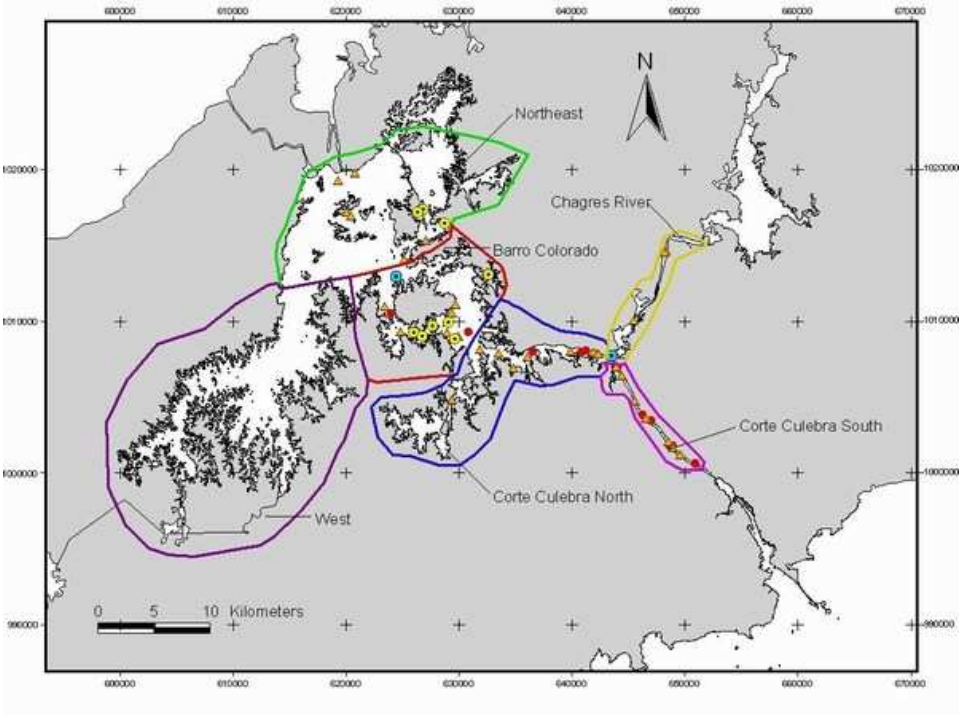


Fig. 3 Map of manatee sightings through boat and aerial surveys per sector.

C. Genetic Analysis

There have been no previous genetic studies on manatees in Panama (Vianna *et al.* 2006, Mou-Sue *et al.* 1990). Sighting manatees in their natural habitat and their skittish response to approaching vessels makes obtaining tissue samples very difficult. In contrast to muscle, bone or blood samples, manatee feces are a particularly useful source of DNA, which makes it relatively easy to collect a large number of samples in a short amount of time. The methodology used in this study represents the first instance in which manatee feces are successfully used as source of DNA, proving to be a completely non-invasive method of sampling.

Giselle Muschet and Mario Gonzalez, a local volunteer, looking for traces of manatee foraging activity and fecal samples for genetic analysis in Bocas del Toro, Panama.

Sampling collection in Bocas del Toro was carried out in the rivers and canals of the San San – Pond Sak (SSPS) Ramsar site March to June 2007. One volunteer from the local community based organization AAMVECONA was trained in sample collection. Each sample was individually handled using sterile latex gloves and introduced into sterile plastic vials. The samples were preserved in 70% ethanol and stored in a plastic cooler containing ice to prevent evaporation during

storage and shipping. The cooler was later exported to Chile where the genetic analyses were carried out (*All samples were collected with permit No.SE/A-9-07. Samples were shipped with the CITES export and import permits No. SEX/A-074-07, and No. 5956, respectively*).

Sampling collection in Lake Gatun was carried out for a total of four days and 20 hours where it was indicated by local people that manatees were commonly seen. Unfortunately no fecal samples were found. However, the personnel of the Aquatic Vegetation Control Unit reported three dead manatees during the course of this study and they managed to collect 3 tissue samples and 2 bone samples from manatees that died of unknown causes during the course of the study.

Genetic analyses were carried out using primers specifically developed for manatees during this study.

DNA was extracted using the QIAmp DNA Stool Mini Kit (QIAGEN ©) following the manufacturer's instructions. The origin and quality of extracted DNA was determined by polymerase chain reaction (PCR) amplification of 503 bp of mitochondrial DNA (mtDNA) control region, initially using primers L15926 and H16498 (Kocher *et al.* 1989). PCR reactions were carried out in a 50µL volume containing 4µL of DNA, 1x reaction buffer, 1.5 mM of MgCl2, 200µM of each dNTP, 0,4µM of each primer, and 1 unit of *Taq* DNA polymerase (Invitrogen ®).

A ~450 bp fragment of the control region of mtDNA was amplified by polymerase chain reaction (PCR) using primers LTMCR01 and HDDCR01 (Muschett *et al.*, in press). PCR products were purified using QIAquick PCR purification kit [Qiagen ®], and were sequenced by Macrogen Inc., Seoul, Korea. The sequences were aligned and the bases were confirmed according to the quality using Proseq v. 2.91 (Filatov, 2002). All sequences were aligned to detect haplotypes using ClustalX v. 1.83 (Thompson *et al.*, 1997), and they were then compared to existing *T. manatus* sequences (Garcia- Rodriguez *et al.*, 1998; Vianna *et al.*, 2006). Arlequin version 2.0 was used to calculate pairwise ΦST between manatee populations from Panama and between Panama and other ten manatee populations from previous studies (Garcia-Rodriguez *et al.*, 1998; Vianna *et al.*, 2006).

Sixty four (64.6%) of the 99 fecal samples from Bocas del Toro, and the 3 tissue samples from Lake Gatun were successfully amplified.. A total of 67 samples were sequenced, and 45 (67%) yielded high quality mtDNA sequences, 42 sequences from fecal samples and the 3 tissue samples. The analysis yielded only one known haplotype for the West-Indian manatee, haplotype J. Considering each sample a different individual, no genetic diversity was found for both populations in Panama ($\Phi_{ST} = 0$; $p = 1.0$). When compared to ten other manatee populations it was found high population structure ($\Phi_{ST} = 0.78$, $p < 0.001$). As expected the Panama population is completely differentiated from Florida, Puerto Rico, Dominican Republica and the ESU from Guyana and Brazil ($\Phi_{ST} = 1$) (Vianna *et al.*, 2006). However, it is less differentiated from Colombia ($\Phi_{ST} = 0.31$), Mexico ($\Phi_{ST} = 0.59$), Belize ($\Phi_{ST} = 0.62$) and Venezuela ($\Phi_{ST} = 0.64$) where the haplotype J is also found but in lower frequency than Panama.

MAJOR PROJECT OUTCOMES

- Thanks to the support of the Rufford Foundation, during the course of this project Giselle Muschett completed her Master's Thesis titled **"Distribution and genetic studies of the manatee (*Trichechus manatus*) in the Panama Canal Watershed"**, and has since graduated from the Master's in Natural Resources in the Pontificia Universidad Católica de Chile. She has now returned to Panama where she is now working for the Smithsonian Fundation to help raise funds for environmental education and scientific programs.

- This project has been an incredible tool for raising awareness about this virtually unknown manatee population in the country. The process of interviews was particularly enlightening and the interaction with local people and volunteers who eagerly reported manatee sightings was one of the highlights of the field work. This "indirect environmental education" served not only to increase the knowledge of manatees but also create a sense of wonder that such large creatures could exist in such a transited body of water and go "undetected". It created in many people a sense of stewardship for these incredibly charismatic creatures.

The Project benefited from the help of many volunteers such as the members of the community based organization AAMVECONA in Bocas del Toro.

- Interaction with local people both in and around the Panama Canal, and in Bocas del Toro yielded much information that was previously undocumented. This included places where manatees usually congregate, and the fact that many members of the community based organization AAMVECONA in Bocas del Toro could easily identify manatee feces. This simplified the training process in collection of fecal samples for genetic sampling. In addition, interviews revealed that hunting of manatees does not represent a major threat to the Canal population as most interviewees agreed that the main causes of death could be attributed to collisions with boats.

- International exposure of the project was an unplanned result. Since the project was carried out in Panama and Chile simultaneously, manatee

conservation issues were presented in both countries during the course of the project.

- Prior to this study the methodology for aerial surveys had never been attempted with a helicopter. Its smaller size and better maneuverability allowed for excellent surveys of the lake. The commercial pilot has now been trained in manatee transect surveys.

Presentation of results and delivery of manuscripts to the National Environmental Authority (ANAM) and AAMVECONA volunteers allowed for the transfer of knowledge to local people and authorities.

- This study represents an innovation in manatee research, pioneering a new non-invasive methodology for genetic sampling. Fecal samples had been used successfully for genetic analyses in other marine mammals, but this is the first time it has been successfully used on the Wes-Indian manatee. In addition, this is the first time that manatee specific primers were developed.

- The results obtained here indicate that there is no genetic variation in Panama manatees. Using mitochondrial DNA analyses all samples yielded a single haplotype, Haplotype J. This is very worrisome for Panama's manatees. The population in Bocas del Toro particularly indicates a recent past bottle neck, due probably to hunting and habitat degradation, and subsequent reproduction from a few related females. Then in 1964 individuals from this highly homogenous population were relocated to Lake Gatun, where the founder effect was probably intensified. These results highlight the dangers of carrying out relocation programs without proper genetic and ecological studies prior to the activities.

RECOMENDATIONS

- Further studies targeted at categorizing the abiotic and biotic factors that can influence manatee distribution in Lake Gatun are needed. Factors such as boat traffic, presence of aquatic vegetation, and undisturbed forest on land seem to influence manatee site choice.

- Further studies on the manatee's genetic composition are also recommended. Since mtDNA does not allow for the identification of individuals the results presented here must be handled with caution. The use of nuclear NDA and microsatellite markers would allow the identification of individuals.

PUBLICATIONS

2009

1) Muschett, G., Bonacic, C. and Vianna, J. 2009. A non-invasive sampling method for genetic analysis in the West-Indian manatee (*Trichechus manatus*). Marine Mammal Science *in press.*

De: onbehalfof@scholarone.com en nombre de mmsci@megalink.net
Enviado el: sáb 07/02/2009 12:16
Para: Juliana Vianna
CC: marinemammalscience@gmail.com
Asunto: Marine Mammal Science - Editorial Decision for MMSCI-2696.R2

07-Feb-2009

Dear Miss Vianna:

I am very pleased to inform you that your revised Note entitled "A NON-INVASIVE SAMPLING METHOD FOR GENETIC ANALYSIS OF THE WEST-INDIAN MANATEE (Trichechus manatus)" has been accepted for publication in Marine Mammal Science. I am attaching a copy of your manuscript with a few minor editorial changes that should be made in your final text file. Once the final manuscript and figures are prepared, please submit them by e-mail to marinemammalscience@gmail.com.
[… … …]
The final files will be forwarded immediately to Assistant Editor Gary Smith (gjsmith@rogers.com) for copy editing. Mr. Smith will contact you with any queries regarding inconsistencies in your manuscript. You will receive an e-mail notification when your proofs are ready (about six weeks from when we receive your final text and figure files). This e-mail will include a link to the site where you can download your proofs. […].

Congratulations and thank you for sending your manuscript to Marine Mammal Science.

Sincerely,
Daryl J. Boness
Editor
Marine Mammal Science

2) Muschett, G and Vianna, J. *in prep.* Abundance and distribution of the West-Indian manatee (*Trichechus manatus*) in the Panama Canal.

2008

Muschett, G. 2008. **Distribution and genetic studies of the manatee (*Trichechus manatus*) in the Panama Canal Watershed.** Master's Thesis. Natural Resouces Master's Program. Pontificia Universidad Catolica de Chile. Ave. Vicuña Mackenna, 4680, Macul, Santiago, Chile.

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www.ruffordsmallgrants.org

WILDLIFE TRUST
WILDLIFE • ECOSYSTEMS • HUMANS

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PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE

ACP
AUTORIDAD DEL CANAL DE PANAMA

UNIVERSIDAD ANDRES BELLO

Smithsonian Institution

AAMVECONA
ASOCIACIÓN DE AMIGOS Y VECINOS DE LA COSTA Y LA NATURALEZA

GAMBOA
RAINFOREST RESORT

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VIANNA, J.A., BONDE, R.K., CABALLERO, S., GIRALDO, J.P., LIMA, R.P., CLARK, A.M., MARMONTEL, M., MORALES-VELA, B., DE SOUSA, M. J., PARR, L., RODRÍGUEZLÓPEZ, M.A., MIGNUCCI-GIANNONI, A. A., POWELL, J. AND SANTOS, F. R. 2006. Phylogeography, phylogeny and hybridization in trichechid sirenians: implications on manatee conservation. Molecular Ecology 15: 433-447.

EXPENSES

Exchange rate averaged over the project: US$ 1 = £ 0.490

DESCRIPTION	Budgeted from RSG (£)	Amount Spent (£)		Other sources (£)	Total Expenses
I. Manatee distribution and habitat use					
A. Field Equipment (e.g. camera, GPS)	400	274		155	
B. Field work (interviews, boat, aerial surveys)	1,680				
Aerial Surveys		971		321	
Food		489		424	
Car fuel for interviews		228			
Complimentary material for interviews (e.g. maps, printing supplies)		41			
Permits (collection, CITES, legalization)	80	97			
Cartography (maps)	320	245		197	
Subtotals	***2,480***	***2,345***		***1,097***	***3,442***
II. Genetic Analyses	1,800				
Sample Collection (food and lodge, boat rental)		196		311	
Laboratory Materials		312			
Sample analysis		495		352	
Transport export samples to Chile		155			
Travel/airfare (one roundtrip ticket to Chile)		642		825	
Subtotals	***1,800***	***1,800***		***1,488***	***3,288***
III. Dissemination of results	720				
Travel/airfare (e.g. Bocas del Toro, food and lodge)		393			
Printing costs		149		78	
Communications	0	150		63	
Equipment rental	0	156		80	
Subtotals	***720***	***848***		***221***	***1,069***
TOTALS	**5,000**	**4,993**		**2,806**	***7,799***

*****Note: additional funding was obtained through Wildlife Trust Alliance, Andres Bello University and the Catholic University of Chile.*

Budgeted vs. Actual Expenditures

Many factors that contributed to the differences between budgeted and actual expenses, the main one being the difficulty of carrying out the project in two separate countries and foreseeing expenses in both nations. Though this approach allowed the international exposure of the project through presentations in both Chile and Panama, the exchange rate of the Chilean peso was highly variable throughout the project and exact exchange rates were difficult to compute. Fuel prices also reached to record highs during the course of 2008 and affected the budget negatively.

There were however some positive impacts that allowed savings in other budget items, such as donations of field equipment (e.g. GPS) and cartography services through volunteers and friends of the project. One major and unexpected positive impact on the budget was the low cost of sequencing of DNA samples thanks to technology developed in Macrogen, Korea, which resulted in savings of almost one half of the original proposed costs for that item.