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[ECOLOGY, GENETICS AND CONSERVATION OF MANATEES IN FRENCH GUIANA]

Proposal

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1 BACKGROUND

Antillean manatees *Trichechus manatus manatus* are aquatic mammals considered endangered according to the IUCN Red List of Threatened Species (Self-Sullivan y Mignucci-Giannoni 2008). Manatees face similar threats throughout their range, which include habitat degradation and loss, watercraft collisions, incidental catch/accidental take in fishing gear, pollution, human disturbance, natural disasters, and hunting (Quintana-Rizzo y J.E. Reynolds 2007).

The National Park and other entities in Guadeloupe (France) are interested in re-establishing manatees in the waters of the natural reserve called “Grand Cul-de-Sac Marin” The species has been extinct in the waters of Guadeloupe for a number of decades, having been wiped out locally by hunting (Reynolds y Wetzel 2008). The reintroduction project aim to overcome and reverse loss of biodiversity in Guadeloupe, to improve the global conservation status of the species and subspecies, and to provide a transferable model for other conservation projects (Parc National de la Guadeloupe 2009).

In French Guiana (FG), an administrative unit of France, preliminary interview surveys suggested that manatees are still present and regularly sighted all along the coast and up to 80 km inland, but may be less abundant than in the recent past (de Thoisy et al. 2003). As the alluvial coastal plain is quite narrow, their habitats are naturally restricted. The manatees are also encountered on nearby rocky shores (Cayenne Island, Malmarouny, Ilet-la-Mère and Rocky point at Kourou) and up to 80 km upstream in some of the larger rivers as the Maroni, the Approuague and the Oyapock. The relatively pristine state and quietness of some estuarine areas may play a

major role in the suitability of habitats for manatees (Artigas *et al.* 2003). Direct threats, such as hunting for meat and for local customs, appear to occur at only low levels. There is a general disinterest in hunting amongst younger people, and the traditional sacred status of the species may help to prevent illegal hunting in some areas (de Thoisy *et al.* 2003). At the moment, their secretive behaviour, fairly undisturbed estuarine habitats, and protection laws create an optimistic outlook for the future of the manatees in French Guiana (Artigas *et al.* 2003), and could be a plausible "parental" population for the reintroduction project of Guadeloupe.

However, the knowledge on the local population of manatees is far to be enough in order to take such a decision. Some specific questions have to be answered in this regard (Adapted from Quintana-Rizzo y Reynolds 2010).

- A. **What is the current distribution and habitat use of manatees in FG?** Based on interviews, it appeared that the manatees are still present all along the coast and in most estuaries of FG (de Thoisy *et al.* 2003). During a preliminary excursion, I was able to confirm the presence of manatees in most of the visited areas (Castelblanco-Martínez 2012). It is necessary to validate/update such information with a systematic study, involving an intensive field work. Also, manatees may move between FG and parts of Brazil and Suriname (Quintana-Rizzo y Reynolds 2010). A radio-tagged study could help to identify whether those movements occur.
- B. **What is the minimum population size of the manatee population inhabiting FG?** Removal of manatees from smaller populations could create undue and unacceptable risk for those groups. If the FG population is estimated to be fewer than 500 individuals, probably this would not be an appropriate population from which to remove individuals (Reynolds y Wetzel 2008). During our preliminary visit, we confirm the presence of the species in most of the places with relatively

little effort. For instance, in Kaw River we sought at minimum three different individuals, counted during the first work-day (Castelblanco-Martínez 2012). It seems reasonable to assume that a bigger effort will produce a bigger number of manatee encounters, and so, a better estimate of number of individuals. Due to the very difficult environmental conditions, it would be impossible to have an actual population size value. Nevertheless, we could be able to determine the minimum number of individuals, and perhaps, the areas with more number of individuals. Once again, a detailed study about distribution and abundance is needed.

- C. How much subpopulations can be considered, what is their diversity and genetic proximity? Do the manatees proposed for removals exhibit genetic variability in genes undergoing active selection? What is the prevalence of hybrids?** In previous studies, scientists found that three individuals from FG (100% of the samples), and one from northern Brazil, identified as *Trichechus manatus*, presented haplotypes that are affiliated with those of the Amazonian manatee *T. inunguis* (Vianna et al. 2006). Garcia-Rodriguez et al. 1998 (1998) also found three specimens from Guiana with the same characteristics. The authors specifically advise against translocation of animals from these areas to areas where the hybridization was not genetically detected. Allowing or introducing new regions of possible hybridization would be very costly for conservation efforts. It is mandatory to develop a study that allows us to assess the genetic structure of the FG population, and to guarantee that the prevalence of hybrids is not putting in risk the population viability.
- D. What is the current conservation status of *Trichechus manatus* in FG? What are the current threats to the population? Are they increasing, decreasing or under control?** If poorly controlled threats are killing or reducing health and

reproductive potential of manatees in an area, removals could exacerbate an already precarious situation (Reynolds y Wetzel 2008). It is not recommendable to remove manatees from populations for which threats are serious and/or uncontrolled. We did not see any important threat to manatees in FG during our first visit. In general, the area looks very well conserved, without any evident drastic sign of pollution, degradation or contamination. The boat traffic is scarce, and it consists mainly in boats of small engines and canoes. The fishing activities recorded are not harmful for manatees. The amount of tourists was relatively low, but the peak of tourism is in dry season, and more visitors are expected in the areas. My first impression was that, in comparison with other areas where manatees are distributed, FG seems to offer a good quality habitat for manatees. In despite of this, manatees are apparently hunted in some areas (Benoit com. pers.). A systematic study has to be done, in order to describe and valorize the potential and actual risks for manatees in the territory.

- E. **What is the health status of the local population?** Bacterial and viral screens should be run to assess whether manatees from FG seem to carry infections that could debilitate individuals or be spread through the population. Body burdens of contaminants should also be assessed and compared, as possible, to toxic equivalent values. In addition, studies of biomarkers of exposure and effects should be done to assess actual responses of individuals to environmental insults (Quintana-Rizzo y Reynolds 2010). Studies assessing the potential impact of contaminants on manatees and other marine life are also necessary. In FG, bodies of water are being contaminated with fuel as a result of common practices like emptying fuel tanks in the water. Additionally, rice fields may have negative impacts on aquatic ecosystems because insecticides such as DDT, dieldrine, and aldrine may still be

used (de Thoisy et al. 2003). In FG, contaminants have been assessed in other species of marine vertebrates (Guirlet *et al.* 2010) but, so far, there are not studies about the health status of manatees in the territory.

If these questions are answered properly, a very positive outcome (independent of whether the reintroduction itself actually occurs) would be vastly improved knowledge of the status of manatees and threats to manatees in certain locations in the Wider Caribbean (Quintana-Rizzo y Reynolds 2010).

Governmental and non-governmental institutions in FG are extremely enthusiastic and supportive with the research and conservation of manatees; and have done relevant accomplishments in this regard. To guarantee the success of the project, it is necessary to count with the approval, involving and participation of local entities and population; in all the process's phases. They also have the necessary knowledge on the territory and resources to perform a field work at high quality standards.

2 OBJECTIVES

Main Objective

To evaluate the current status, distribution, abundance, threats and viability of the Antillean manatees population in French Guiana

Specific Objectives

- ❖ To determine the effective distribution area for the manatee population in FG, habitat use and the level of connectivity between subpopulations using complementary methodologies.

- ❖ To assess the minimum population size of the manatee population inhabiting FG; and the proportion of calves.
- ❖ To determine human and natural factors that threatens the survival, health, or reproduction of manatees.
- ❖ To establish genetic diversity, population structure, prevalence of hybrids and significant evolution units in the territory.
- ❖ To infer population trends and the biological effect of removing individuals on the population success.

3 AREA OF STUDY

French Guiana, the largest French over-sea department, is situated on the north-east coast of South America between 2° - 6° latitude North and 51° - 55° longitude East (see Map 1). It belongs to the equatorial zone of the northern hemisphere. The border with Surinam is marked by the Maroni River, the French Guiana-Brazil border by the River Oyapock (Fig. 1).

Marine biodiversity in French Guiana is strongly influenced by the amagon River waters of the river Amazon, which constitute a major structuring factor for the estuarine, coastal, and shelf marine ecosystems. Moreover, the marked seasonal and interannual variabilities play important roles in the stability or fluctuations in the environmental parameters that influence biodiversity at the ecological, population, and genetic levels (Artigas *et al.* 2003). The climate is equatorial and therefore characterized by a wet and a dry season, and a maximal variation of the average temperature of 2° C over the year (Spiegelberger 2002). There are 2 periods of high rainfall, May - June (the principal one) and January- February (secondary). The dry season lasts from July to December when stable south-eastern trade winds are dominant. The flow of the rivers of

French Guiana is similar to that of the rainfall being maximum in May-June and minimum generally in November, when the flow rate of the Amazon River is considerably lower. The tide in French Guiana is semidiurnal, with amplitude of up to 2.5 meters (spring tides, mesotidal regime). The coastal waters are highly turbid, and extensive mudflats occur and migrate along the coast (Artigas et al. 2003).



Figure 1. French Guiana

In Coswine swamps, water parameters were similar to those described in other studies: water depth varied from 2.5 m to more than 20 m; water temperature was between 24.5°C and 30.3°C and pH varied between 5.5 and 6.9. Salinity was low (0.0‰ to 1.3‰) with 86.9% of all samples taken in fresh water. No submerged aquatic vegetation was found in the study area. A botanical survey along the banks revealed that

most plants seem to be potential forage for manatees. Red Mangrove is very abundant throughout the area. It is suggested, therefore, that manatees graze on the bank vegetation, where feeding traces were found, or leave the area to feed (Spiegelberger y Ganslosser 2005).

4 METHODOLOGY

In French Guiana, manatees are distributed mainly in coastal environments, estuaries, rivers and floodplains characterized by low water transparency, forest canopy and water level changes. These facts, and the secretive behavior of the manatee, have made difficult to follow and observe the species.

Our goals are several and diverse, and different methodological approaches are necessary in order to reach them (Table 1). The first phase of the project is designed to develop noninvasive methodologies, to achieve the objectives of assessing distribution, habitat use and population size. These methods include boat and land-based surveys, using of Side-Scan Sonar, observation of feeding tracks and, if possible, collection of fecal samples. Also, interviews will be performed to complement former information.

The second phase has to be designed on the basis of the results obtained during the first phase. This part of the project aim to capture manatees for health assessment and sample collection, and if possible, instrument some individuals to track them by telemetry techniques. Therefore, it will yield information about genetics, biology history and movements of the manatees in French Guiana.

Table 1. Methodologies (in rows) proposed to reach the goals (in columns), with more (●) or less (○) confidence. For example, ‘direct visual surveys’ can yield confident results about ‘distribution’ and ‘minimum population size’. The method also can give some clues about ‘habitat use and connectivity’; but does not allow us to infer conservation status, genetics, health and feeding habits. Note that no method is enough to reach all the goals proposed.

		GOALS						
Methodology		Distribution	Habitat use and connectivity	Minimum Population Size	Conservation Status	Genetics	Health Status	Feeding habits
Noninvasive	Direct Visual Surveys	●	○	●	●			
	Side Scan Sonar	●	○	●				
	Bioacustics	●						○
	Indirect evidences (feces)	●	○			●	○	●
	Indirect evidences (feeding tracks)	●	○					●
	Interviews	○	○		●			○
Invasive	Direct sampling (capturing)	●		○		●	●	●
	Telemetry	●	●					○
	Isotopes	●	●					○

4.1 Noninvasive methodology

It is proposed to use a multi-approach methodology to detect and count the individuals. All this methods are noninvasive, it means that minimize disturbance to the animal. The distribution region will be divided in eight main areas, covering islands, estuaries and rivers (Fig. 2). Those areas are located in altitudes lower than 200 meters above sea level. In total, we will cover all the coastal area of French Guiana (aprox. 330km of coastline), and up to 80 km upstream the rivers. The proposed areas could be modified after a first incursion to the field.

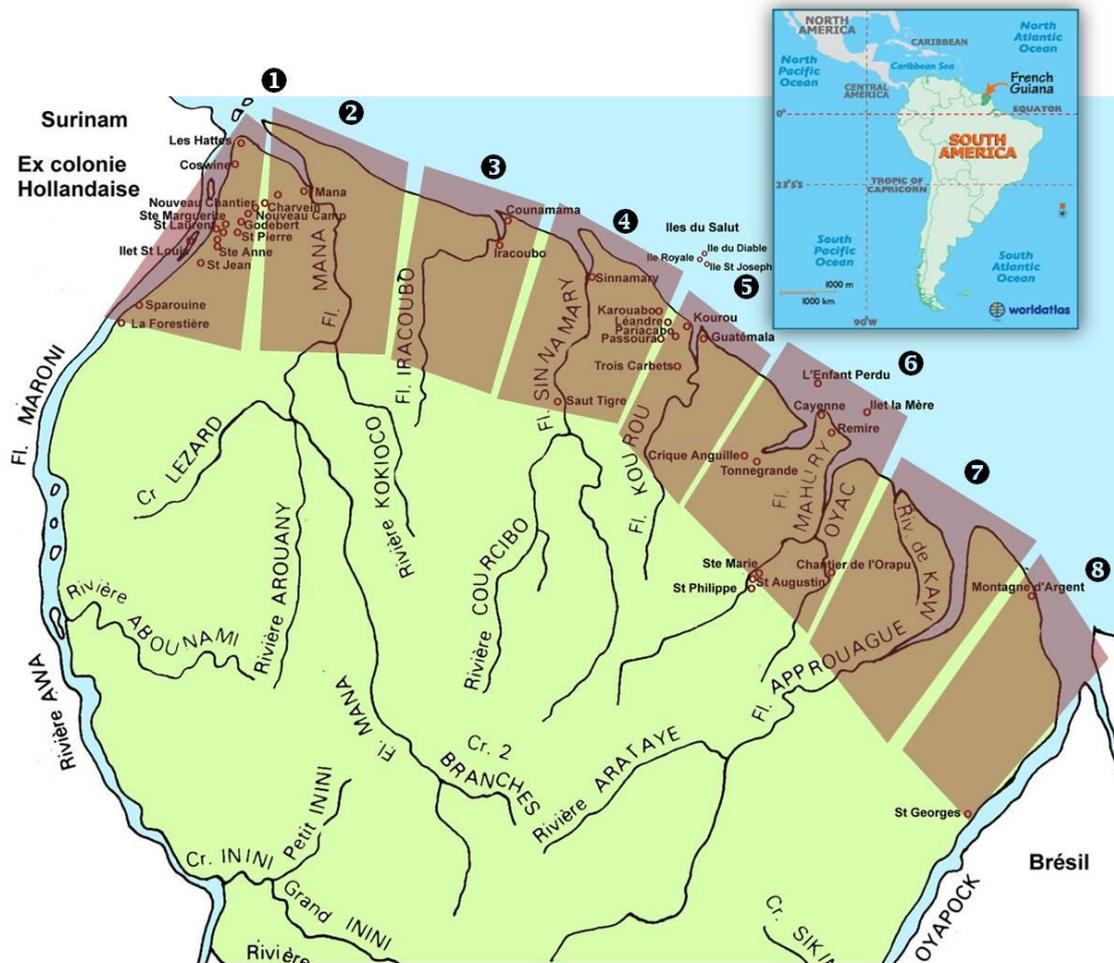


Fig. 2. Areas of survey in French Guiana. 1) Maroni, 2) Mana, 3) Iracoubo, 4) Sinnamary, 5) Kourou, 6) Cayenne, 7) Approuague and 8) Oyapock

Each area will be treated separately, but following the same field collection methods and the same effort in time. Protocols to determine distribution and abundance of manatees will be applied in the different areas simultaneously, depending on the logistic circumstances. Field surveys will be carried out during dry season, when manatees are concentrated in some parts of the watercourses or lagoons. Also, the low pluviosity will increase the effective field time. Information obtained will be organized in databases and maps.

A. Observations from a fixed point (shore or anchored boat)

There are specific techniques that observers can use to determine and record the number of sirenians encountered in a particular field. Keeping track of individual breathing patterns can help determine the number of manatees in a location (Aragones *et al.* in press). However, this technique is not recommended for large areas, so its application will be limited to special cases, for example to establish size group of manatees restricted in deep and relatively small areas; which is a common case in estuaries and rivers in FG. Factors that may impact sighting conditions will be registered as they are very important for examining variability in detection probability and possible detection bias. Manatees usually breathe simultaneously when in a group, and the Maximum Number of Simultaneous Sightings (NMSS) will be assumed as the number of manatees counted at the surface at one time. Number of sightings per hour and NMSS will be used as relative indices of abundance (Castelblanco-Martínez *et al.* 2009).

On the basis on preliminary knowledge about manatee distribution in FG (de Thoisy *et al.* 2003), quick but highly efficient surveys in hotspot areas were established in order to detect manatees and to determine the relative abundance of individuals (Fig. 3). These hotspots can be modified or new hotspots may be identified during the field work. The hotspots correspond mainly to estuaries or river mouths; or high points in beaches or islands.



Figure 3. Preliminary hotspots of manatee presence in French Guiana.

B. Boat-based surveys. Assessing the absolute (a count of the number of individuals per unit area) or relative (an estimation of population size) abundance of sirenians using boat based surveys is difficult but possible. Through line transect sampling along a predetermined track, a researcher can determine the density of a population by counting objects (individual, group, or cue) and measuring their distance from the observer. The density measurement is then based on the determination of the detection function, defined as the probability of detecting an object, given that object is at a specific distance from a random line or point. Once density is measured, estimates of population size can be determined and monitored (Aragones *et al.* 2012).

As a modification of this method, a relatively new technique has been successfully used to detect and count manatees in environments similar to that of French Guiana: The side scan sonar. Side-scan sonar is a valuable tool that can assist scientists and managers in documenting the distribution and habitat use of manatees in complex waterways, especially in freshwater systems (Gonzalez-Socoloske *et al.* 2009). It could be also used to establish relative index of abundance if the procedures are well

standardized (Arévalo-González et al. in prep.). It is proposed to use Side-scan sonar as a tool to detect and count manatees in specific areas. Since the best images are produced in calm or moderate waters (with small waves or minimal currents), traveling in a linear direction at low speed, this method will be used in specific areas as well. Investigators will search for manatees using a Humminbird 997 SI COMBO side-scan sonar (SSS) on linear transects at a rate of 4 km per hour. The sonar technology is based on the use of sound waves to detect and define structures and characteristics of the substrate such as contour, composition and depth. This tool uses a submerged element (transducer) towed by the boat, which emits a sonar signal directed towards the bottom and receives the corresponding echoes. The signal is then passed on to a computer, which transforms it into a digitized image on a screen, displaying a continuous reading (Gonzalez-Socoloske y Olivera-Gomez 2012). We will try to identify the manatees considering the characteristics of the shadows produced by the objects. Images obtained will be filtered and selected, and abundance and density values will be obtained through the Distance software. It would be necessary to carry on a previous workshop with at least one specialist in Side-scan sonar along the investigators to learn the correct use of the sonar, and therefore diminish bias caused by wrong implementation of the method.

C. Indirect records. Indirect records are understood as signals of the manatee presence like feeding signs or excrements. Observations will be made of typical manatee feeding signs on leaves and stalks on macrophytes communities, which are easily distinguishable by experienced observers. As well, fecal samples found floating will be collected (see D. Samples collection). It is not recommended to use indirect records as abundance indexes, as it is difficult to assess the number of individuals. However, this information is extremely useful to determine occurrence of manatees, and therefore, their distribution.

D. Interviews and interaction with local community. Interviews with inhabitants that shared habitat with manatees, especially fishermen, will be developed. Depth-interviews will be designed in order to achieve information about distribution areas, connectivity, abundance and population trends. We will seek to assess the current conservation status of the population and main local threats.

E. Habitat characteristics and human presence. The record of the physic and chemical characteristic of the manatee habits are important to establish the preferred conditions for the species, and therefore, infer potential areas of distribution. Also, it allows us to evaluate the conservation status of the ecosystem. During all the surveys ecologic characteristics will be recorder, as well as the human presence in the area. Fishing activities and intensity of boat traffic will be register.

D. Samples collection. During field work we will attempt to collect samples like material donated by fishermen (bones, skin), fecal samples or skin samples from manatees in captivity. Each sample was handled individually 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. DNA will be extracted using the QIAmp DNA Stool Mini Kit (QIAGEN) following the manufacturer's instructions. The origin and quality of extracted DNA will be determined by polymerase chain reaction (PCR) amplification of 503 bp of mitochondrial DNA (mtDNA) control region, using the appropriate primers (Muschett *et al.* 2009). Those samples will be analyzed in order to establish the phylogenetic structure of *T. manatus* for FG. For this, a molecular sex identification will be developed (Tringali *et al.* 2008), and two types of molecular markers: the DNA mitochondrial control region and microsatellites.

Fecal samples have will be preserved and stored in 70% ethanol (Colares 1990) or dried as appropriate. By comparing the vegetal fragments with the voucher microscope slides, or illustrations in the reference collection, we will identify the source of the fecal material. The slides will be scanned qualitatively to determine which items are present (Heinsohn y Birch 1972). The relative volumes of the items will be estimated by means of a quantitative technique following Marsh et al. (1982). Also, feces can be useful to determine the reproductive status of the individual by hormone assessment (Larkin et al. 2005).

The techniques used to record manatees, indirect evidences and habitat characteristics depend on the type of environment (Table 2).

Table 2. Type of surveys that will be employed depending on the type of environment (beach, estuary or river).

Environment	Type of survey	Description	Recommended time of survey	Type of information possible to obtain				
				Visual records	Sonar image records	Feeding tracks	Ecological characteristics	Human presence
Beach	1	From the shore, using high points with a wide view of the area	60min	●			●	●
	2	From a stationary boat	60min	●			●	●
River	3	From a stationary boat	60min	●			●	●
	4	From a small boat drifting with its engine off (average speed 4.0 km h ⁻¹)	60min	●		●	●	●
	5	From a small boat at low speed using a Side Sonar (average speed 5-10 km h ⁻¹)	variable	●	●		●	●
		From a small boat at medium speed (average speed 40 km h ⁻¹)	variable	●		●	●	●

4.2. Invasive methodology

Some information about the ecology and biology history of the manatees only can be collected through invasive methods. Manatees have to be captured and handled in order to collect samples for genetic studies, health assessment or tagging with a telemetry devise.

During these interactions, qualified scientists and veterinarians will assess the overall health of the manatee. An animal's general appearance, including skin condition, body condition, girth and other morphometrics, and dorsal subcutaneous fat thickness, will be assessed to provide indicators of individual health and condition. Moreover, the evaluation of manatee health based on monitoring of physiological parameters such as oral temperature (OT) (as a proxy for core body temperature), heart rate (HR), and respiration rate (RR) will be defined (Wong et al. 2012).

Another important issue that could give us a better idea of the population structure, is the home range and movements of the manatees into the territory, and if so, to the neighbor countries. Telemetry is a useful method to determine the location and movement of wild manatees in order to obtain sufficient reliable information for interpreting behavior (Michelson 1981, Best *et al.* 1981; Rosas y Pimentel 2001, Martin y da Silva 1998, Reid et al. 1995, Arnould y Hindell 2001). The type of environment of French Guiana represents a challenge for manatee tracking, due to the flooding vegetation and complexity of the river systems. However, some successful experiences in Brazilian and Peruvian Amazon can be used as a basis. In Brazil, Amazonian manatees were radiotracked with an Advanced Telemetry Systems (ATS) transmitter (Advanced Telemetry Systems Inc., Isanti, MN, USA) on the 164MHz band using a three-element Yagi antenna. They were caught under government permit by research

teams, sometimes aided by local hunters, mostly using a wide mesh net. Attempts were made to locate each individual once a day (Arraut *et al.* 2009).

Invasive methods represent an important economic and human investment, but the robustness of the results make worthy that effort. So far, no manatee capture has been attempted in French Guiana with scientific purposes. The first phase of the project will be supposed to yield enough information in order to know the best places to carry on manatee captures. Therefore, it is proposed that simultaneously to the noninvasive methods phase, the researcher team should participate in manatee captures and telemetry projects developed in other parts of the distribution area (Florida, Mexico, Belize, Brasil, Puerto Rico) to learn the techniques and procedures.

5 EXPECTED RESULTS

This research project will allow an evaluation of distribution and current status of *Trichechus manatus* in French Guiana and to evaluate realistic conservation issues. It is hope that basic knowledge gaps on the manatee situation can be filled through this proposal. Using the information about minimal number of individuals, we will be able to develop a Population Viability Analysis in order to evaluate the impact of extracting individuals from this population. Also, the project aims to start a capture program, in order to assess the health conditions of the manatees, to record some biology history aspects, and tentatively, to radiotrack some individuals.

6 OUTPUTS

- Report on the realistic evaluation of the consequences of individual extraction process on the natural population viability.

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- An article about size, distribution and conservation status of Antillean manatee population in French Guiana.
 - An article about genetic diversity and populational structure of Antillean manatees in French Guiana
 - Determination of historical and current threats on manatees and their habitats as a basis for further conservational management plans.
 - A Population Viability Analysis in order to detect the trends of manatee population in French Guiana.
 - Presentation at international conferences and meetings, pre review scientific articles, etc.
 - To identify hotspots areas for manatee populations, and to propose a management plan for those areas.

7 TIMETABLE

	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL
First team reunion	■											
Workshop "use of side-scan and other techniques"	■	■										
Visit to other capture/telemetry programs	■	■	■	■	■	■						
Adjusting methods in field		■										
Boat and land surveys			■	■	■	■		■	■	■	■	
Interviews			■	■	■	■						
Second team reunion							■					
Preliminary report							■					
Adjusting capture methods							■	■	■	■		
Capturing/telemetry program							■	■	■			
Genetic Analysis					■	■	■	■	■	■		
Third team reunion										■		
Preparation of final report									■	■	■	■
Conclusive reunion												■

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