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The Detection of Amazonian Manatees (Trichechus inunguis) Using Side-Scan Sonar and the Effect of Oil Activities on Their Habitats in Eastern Ecuador

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THE DETECTION OF AMAZONIAN MANATEES (*TRICHECHUS INUNGUIS*) USING SIDE-SCAN SONAR AND THE EFFECT OF OIL ACTIVITIES ON THEIR HABITATS IN EASTERN ECUADOR

By

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ABSTRACT

Substantial hunting pressure and habitat destruction caused by oil extraction has critically endangered the Amazonian manatee in Ecuador. The current population status is unknown because an effective method to observe them in the wild has yet to be developed. This study explored whether the Amazonian manatee persists or has been extirpated in the eastern Ecuadorian Amazon utilizing side-scan sonar to increase odds of detection. Spatial differences in probability of detection were quantified if manatees were observed. The level of chemical contamination was determined and compared spatially and temporally against historical data. Data were collected using opportunistic transect surveys and grab sampling of surface water in Yasuni National Park, Lagartococha, and Cuyabeno Wildlife Reserve. Surveys confirmed that the manatee population is extant. Manatees were encountered more often in Cuyabeno Wildlife Reserve than in Lagartococha and Yasuni. Side-scan sonar detected more manatees than previously reported in 1996-1999. Side-scan sonar is a viable method for detection of manatees in the Ecuadorian Amazon system and resulted in greater detection as a function of effort. All future population studies should incorporate side-scan sonar. Lead, arsenic, mercury, polynuclear aromatic hydrocarbons [PAHs], and volatile organic compounds [VOCs] were not detected in the waters of the study region. High total petroleum hydrocarbon [TPH] levels were measured in 7 samples from Yasuni National Park. The concentrations of TPH were higher in Yasuni National Park than in Lagartococha and Cuyabeno. TPHs were detected only in the study region with a recent oil spill; there was no evidence that TPHs were higher near oil production wells and pipelines. The concentrations of TPH were significantly different than those measured in 1998 ($z = 3.01710$, $p = 0.0026$). A dedicated study should be performed to develop a protocol for monitoring persistent oil contaminants in the Ecuadorian Amazon and determine their sink.

Keywords: Cuyabeno Wildlife Reserve, Yasuni National Park, Lagartococha, manatee population survey, elusive megafauna, surface water pollution, environmental assessment, persistent petroleum contaminants

DEDICATION

I dedicate this thesis to Edward O. Keith, Ph.D., who devoted his life to research and education and left this world during my journey – without his continuing vision, support, and mentoring I would not be who I am today and the valuable research contained in this thesis would not have been possible; to my Mom, Mary—amazing role model of female scientific intellect, and Sister, Madeline, who were always there to love, encourage, guide, and believe in me, even when I doubted myself; to my best friend, Yesenia, who was there to listen to my complaints and frustrations even when she had no idea what I was talking about—always responding with “you are Superwoman!”; to Grandma Sally and Grandpa Fred, for their love and financial support; to Mr. Fred Fotsch—my high school chemistry teacher, who introduced me to the rewarding world of marine biology. Words cannot express the gratitude I hold in my heart for these beautiful souls, my extended family. The staircase I climbed to complete this project was full of innumerable long, unexpected, educational, and difficult steps. You have all been a part of this unbelievable journey through the Amazon leading to the discovery of my soul and my role in scientific research.

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For their essential experience and guidance in the field work, I thank Victor Utreras and Galo Zapata Rios, M.Sc. from the Wildlife Conservation Society, Quito, Ecuador [WCSE]. Without their valuable knowledge and assistance, this research project would not have been possible. I thank Utreras and Rios for assisting with obtaining research permits in Ecuador. I thank them for helping us navigate the extensive labyrinth of rivers and tributaries in the Amazon River System in Ecuador. I thank them for providing their familiarity with citizens and knowledgeable native guides, specifically the Kichwa guide "Beli". Finally, I thank Utreras and Rios for providing the WCSE boats and captains, for purchasing the food and supplies necessary to sustain us during the research expeditions, and for their hundreds of hours of paddling.

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For analyzing the water samples collected during this study, I thank Florida Spectrum Environmental Services, Inc., president, Lyle Johnson, and the experienced EPA environmental analysts who work there. Without their discounted pricing to assist

the research project and flexibility in terms of sample receiving, log-in, analysis, reporting, and payment terms, this research project would not have been possible. Not to mention, Lyle Johnson is my boss and at risk of losing my job, he cannot be omitted as a major contributor through his unending guidance and support. Additionally, I acknowledge the contribution of Richard Vicchiarelli, M.Sc., supervisor of the Wet Chemistry and HPLC departments at FSES and close, personal friend. I thank Richard for his experiential advice and wisdom throughout all of the stages of this project.

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NOMENCLATURE

EPA	Environmental Protection Agency
NELAC	National Environmental Laboratory Accreditation Conference
IUCN	International Union for the Conservation of Nature
CITES	Convention on International Trade of Endangered Species
CPPMA	Centro de Preservação e Pesquisa de Mamíferos Aquáticos
SOTE	Sistema Oleoducto Trans-Ecuatoriano
OCP	Oleoducto Crudo Pesado
CESR	Center for Economic and Social Rights
FMRI	Florida Marine Research Institute
WCSE	Wildlife Conservation Society Ecuador
VOC	Volatile Organic Compound
PAH	Polynuclear Aromatic Hydrocarbon
TPH	Total Petroleum Hydrocarbon
MDL	Minimum Detection Limit
PQL	Practical Quantitation Limit
FDEP	Florida Department of Environmental Protection
MCL	Maximum Contaminant Level
BWCC	Black and White Color Contrast

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CHAPTER I

INTRODUCTION

PURPOSE OF THE STUDY

This study explored whether the Amazonian manatee persists or has been extirpated in Eastern Ecuador utilizing side-scan sonar to increase odds of detection. Spatial differences in probability of detection were quantified and compared to previous research if manatees were observed. The level of chemical contamination was determined and the results compared spatially and temporally against historical data. The purpose of this thesis is to report on original research examining side-scan sonar as a viable detection technique for Amazonian manatees. This thesis will report on original chemical analysis of water samples from Yasuni National Park, Lagartococha, and Cuyabeno Wildlife Reserve. The results are examined in the broader context of endangered species monitoring and the protection of their habitats.

In this chapter, I introduce the site specific problem statement defined by previous research. The remainder of the chapter focuses on the species of interest, the Amazonian manatee (*Trichechus inunguis),* and the population in eastern Ecuador. The Vulnerable status of the Amazonian manatee and its threats to survival are discussed. This chapter also introduces the history of the oil extraction industry and environmental pollution in the Ecuadorian Amazon.

In Chapter II, I explore the question of whether the Amazonian manatee persists or has been extirpated in the Ecuadorian Amazon by assessing the population status of the species on a wide scale in Yasuni National Park, Lagartococha, and Cuyabeno Wildlife Reserve utilizing side-scan sonar to enhance detectability. This chapter examines if side-scan sonar is a viable and efficient method for collecting Amazonian manatee population data in complex environments. This chapter presents current manatee survey techniques, the difficulties associated with surveying marine mammals, and how side-scan sonar can be used to compliment visual surveys by increasing detectability. The history of sonar use to detect manatees and the development of the side-scan sonar technique are discussed. Research implementing and testing a side-scan sonar method for

detecting manatees in Ecuador is presented. Manatee observations, spatial and temporal differences in probability of detection, and effort are examined. Chapter II is written for a focused audience who is interested in developing manatee or other aquatic mammal survey techniques for complex or difficult habitats such as the Amazon River.

In Chapter III, I investigate the extent of chemical contamination in the Ecuadorian habitat of the Amazonian Manatee. Chemical contaminants identified as constituents of crude oil are examined. The experiment determines if the levels of chemical contaminants vary spatially or temporally since last measured in 1993 (CESR 1994) and 1998 (Sebastian *et al.* 2001) using a quantitative statistical approach. The results are based on original chemical analyses using Environmental Protection Agency [EPA] methods performed at a National Environmental Laboratory Accreditation Conference [NELAC] certified laboratory on samples obtained from the study areas. Relationships between contamination and proximity to oil wells and pipelines are identified. This chapter also discusses habitat monitoring in relation to endangered species and the effects of petroleum industry development occurring in many South American countries. Chapter III is written for a focused audience who is interested in analyzing anthropogenic threats to the survival of a species, chemical contaminant analysis for petroleum toxins, and conservation of natural resources.

Chapter IV provides an executive summary for use by researchers studying the Amazonian manatee or oil contamination in the Ecuadorian Amazon and for use by people working within governmental and non-governmental organizations who are interested in influencing policy. Management of renewable natural resources in developing countries has been hampered by a mix of socioeconomic and political difficulties that in turn have resulted in insufficient knowledge, limited environmental awareness and education, and limited commitment to conservation (Vidal 1993). In environments such as the study area, it can be difficult for conservation efforts to remain current due to these complications. This is especially true when the amount of data is sparse. The purpose of the Executive Summary is to provide an overview of my results. Continuous monitoring and improvement of methodology can provide a better perspective on manatee conservation and environmental pollution in Ecuador. This

information can be used by policy makers to make better decisions that balance conservation of natural resources and economic development.

STATEMENT OF THE PROBLEM

There are few data and no valid population estimates for the Amazonian manatee because an effective method to observe them in the wild has yet to be developed. The population of Amazonian manatees in Ecuador was first studied from 1983-1986 by Timm *et al.* (1986); they verified the presence of the species via 10 observations and predicted the manatee would go extinct in 10-15 years. Denkinger documented 4 observations from 1996-1999 (2010). More data are needed before a realistic status of the current population can be determined. Once on-going, long-term population data have been collected, statistical analysis and population models can be used to accurately predict the trend of the population and identify preferred rivers and lagoons.

It is important to obtain population data because extensive hunting pressure has reduced the population of the once abundant Amazonian manatee in Ecuador (Timm *et al.* 1986). Since the establishment of the CITES Appendix I in 1973, the Amazonian manatee has been protected both internationally and within Ecuador (Denkinger 2010). The Siona Indians have practiced a self-imposed ban on hunting the mammals since 1977 because they observed dwindling numbers (Timm *et al.* 1986). However, hunting has likely continued and the population is decreasing (Marmontel 2008)

Since the discovery of vast amounts of crude oil underneath the dense jungle in the Ecuadorian Amazon in 1967, the Texaco Gulf Consortium and Ecuadorian government have been extracting and exporting oil for profit (Aaen 2006). The development of this industry in the habitat of the Amazonian manatee poses serious risks to the survival of the species. Oil refineries, extraction spills, and pipeline leakage exposed the region to millions of gallons of crude oil and toxic wastes (Aaen 2006). Studies have documented elevated levels of toxic chemicals throughout the region (Sebastian and Hurtig 2004). Increased road building, construction, and boat traffic are also impacting the habitat of the Amazonian manatee.

To address the problem, we studied whether the Amazonian manatee persists or has been extirpated utilizing side-scan sonar to increase detectability. In addition, this study examined water samples from the lagoons and rivers where the manatee resides for arsenic, mercury, lead, PAHs, VOCs, and TPHs.

BACKGROUND INFORMATION AND ENDANGERED STATUS

Two extant families, two extant genera and four extant species represent the Order Sirenia, today. The Family Dugongidae contains *Dugong dugon* and the Family Trichechidae is comprised of *Trichechus senegalensis*, *Trichechus manatus*, and *Trichechus inunguis* (Cantanhede 2005). The most recently extinct sirenian is *Hydrodamalis gigas* of the Dugongidae – discovered in 1741 and extirpated by 1768 (Turvey and Risley 2006). Dugongs are the only surviving members of the family Dugongidae; they inhabit coastal marine waters from eastern Africa to the Philippines and Palau, and between Australia and Okinawa (Belanger and Wittnich 2008). The three extant species of manatee [West Indian (*Trichechus manatus*), Amazonian (*Trichechus inunguis*) and West African (*Trichechus senegalensis*)] live in the shallow tropical and subtropical coastal waters and rivers of the Americas and West Africa (Vianna 2006).

The Amazonian manatee is a threatened aquatic mammal. The International Union for the Conservation of Nature [IUCN] classified the species as “vulnerable” (Vulnerable A3cd ver 3.1, Marmontel 2008) to extinction because the total population estimate is less than 10,000 individuals and declining. The species is also listed in Appendix I of the Convention on the International Trade in Endangered Species of Wild Fauna and Flora (CITES 2013, Keith 2010). A multitude of anthropogenic causes threaten the survival of the species: hunting, habitat destruction, and incidental mortality from gillnets (Marmontel 2008). Historically in Ecuador, indigenous Amazonian tribes, such as the Siona, have reduced the population of the species through subsistence hunting (Timm *et al.* 1986). Today, Amazonian manatees are listed as “critically endangered” in the “Libro Rojo de Los Mamiferos del Ecuador” (Denkinger 2010, Tirira 2011). Over the last forty years, their habitat has been deleteriously impacted by road and industrial construction, toxic chemicals, and increased motor boat traffic. These negative impacts have been tied

to the development of the petroleum industry in the Ecuadorian Amazon (Asimbaya *et al.* 2004).

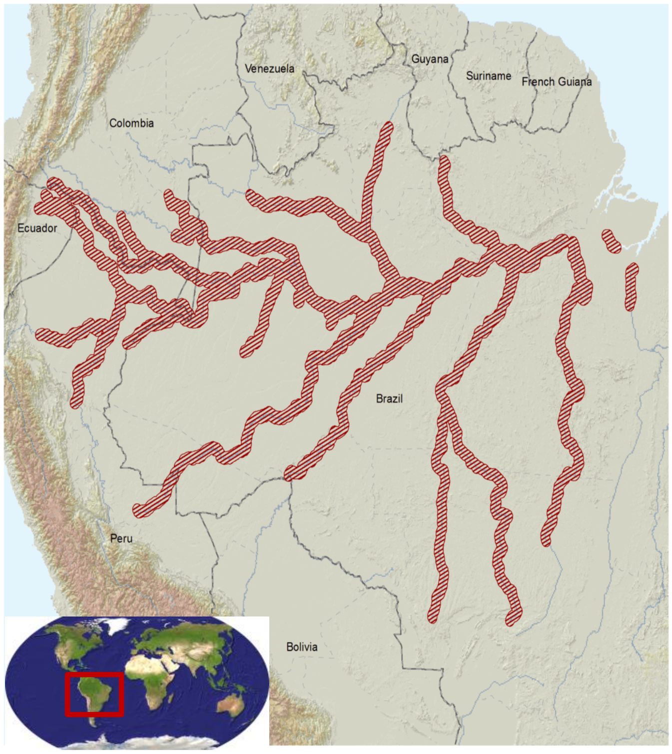


Figure 1: This image is a map showing the geographic range of the Amazonian Manatee. (Marmontel 2008)

The Amazonian manatee, the only exclusively freshwater Sirenian, inhabits the Amazon River basin, including blackwater, whitewater, lagoons and oxbow lakes, in Brazil, Colombia, Peru, and Ecuador (See Figure 1; Marmontel 2008, Colares and Colares 2002, Timm *et al.* 1986). Described by Natterer in 1883 in Brazil, the species is smaller and of more slender proportions than *T. senegalensis* and *T. manatus* with a black body and white markings (Rosas 1994). The Amazonian manatee eats mainly emergent aquatic vegetation (63 species; Arraut *et al.* 2010), especially aquatic grasses and water hyacinth including *Paspalum repens* and *Echinochloa polystachya*. In periods of low water it eats a variety of other plants (Colares and Colares 2002).

Seasons are differentiated by rainfall, rather than temperature, in Amazonia (Arraut *et al.* 2010). The Amazonian manatee migrates seasonally as a response to variation in water level. To optimize foraging, minimize predation, and maximize space,

manatees undergo seasonal migrations (Arraut *et al.* 2010).During periods of high water, manatees migrate to areas with high food availability and low predation. During periods of low water, these areas disappear or become too shallow and may leave manatees more vulnerable to predators.

Manatees in Brazil have been reported to fast during the dry season when the water level drops 10-15 meters, often trapping manatees within deep lagoons and oxbow lakes (Best 1983). With no emergent vegetation accessible and dangerously shallow rivers, individuals conserve energy until the water levels rise again (Best 1983, Gallivan and Best 1986, Arraut *et al.* 2010). Seasonal fasting caused by no available plants has also been suggested in the Antillean manatee during the low water season (Gonzalez-Socoloske 2013). The manatee is ecologically adapted with a low metabolic rate (about 36% of a predicted eutherian metabolic rate based on body size) and stores of blubber (Gallivan and Best 1980). In combination with a reduction in activity, Amazonian manatees may utilize the energy already in their gut contents to fulfill energetic requirements during the initial stages of fasting (Gallivan and Best 1986). This reduces the need to mobilize body energy stores and prolongs its ability to undergo periods of food deprivation (Gallivan and Best 1986).

The gestation period of Amazonian manatees is 12-14 months (Best 1982); they are uniparous and nurse their offspring for 2-3 years (Marmontel 2008). Robin Best (1982) extrapolated breeding seasonality based on data from the lengths of neonates captured during fishing activities in Brazil. The data demonstrated seasonality in births coinciding with rising waters in the region. Giving birth during rising water is advantageous to Amazonian manatees because aquatic and semi-aquatic plant production increases as the water rises. The higher nutritional value of new plant growth benefits both mothers and newborn calves (Best 1982). Florida manatees (*Trichechus manatus latirostris*) also reproduce seasonally, correlated with water temperature fluctuation (Rathbun *et al.* 1995, Marmontel 1995). Most calves are born during the non-winter season (Koelsch 2001); reproductive hormones peak in the spring and/or fall in both male and female captive Florida manatees (Larkin 2000).

The Amazonian manatee is hunted for meat and oil (Wallace 1853, Marmontel 2008). Commercial hunting dates back to 1542 (Rosas 1994) and was most likely the primary cause of severe population declines (Denkinger 2010). In Ecuador, it has been hunted for generations by the Siona Indians (Timm *et al.* 1986). In one hunting method, manatees are caught and killed by driving a wooden plug into the nostrils (Wallace 1853). The carcass, which can weigh up to 450 kg (Amaral *et al.* 2010), is transported to market by canoe. Wallace reported that a hunter would fill his canoe with water, float it below the animal, and then bale out excess water (1853). The use of traditional harpoons is the most widespread hunting method; the use of netting is on the rise (Marmontel 2008). Accurate records of the manatee take by natives are unavailable. One hunter killed between 7 and 10 manatees in an eight month period (Timm *et al.* 1986). Manatees are hunted year round, but are more vulnerable during the dry season where they aggregate in deep lagoons and canals (Denkinger 2010). The manatee has been legally protected since the passage of the CITES Appendix 1 in 1973 and by laws in Ecuador, however, there is little to no enforcement (Denkinger 2010).

Calf mortality is rising due to incidental capture in gillnets used for hunting adult manatees (Marmontel 2008) and fishing gear used for Paiche (*Arapaima gigas)* (Reeves *et al.* 1996). Young animals often drown in the nets; if they survive, they are kept alive for later sale, since young animals have little meat for immediate consumption (Marmontel 2008). A live newborn manatee was confiscated by authorities in Iquitos, Peru on 8 May 1995. The fisherman claimed that it had been caught in fishing net (Reeves *et al.* 1996). These situations are occurring more often with increasing fishing commerce. With the increase in orphaned calves, groups rescue these animals and attempt to rehabilitate and release. Between 1992 and 2005, CPPMA (Centro de Preservação e Pesquisa de Mamíferos Aquáticos) received an average of four calves per year (the numbers increased during heavy drought). Of the 41 calves rescued, 23 (56%) were caught in gillnets, but only four accidentally, while the others were caught in nets set up to catch them with the intent to sell, and even to catch on request (Marmontel 2008). Most of these calves, even when rescued, die in captivity or after they are released (da Silva 2011 oral presentation). This is detrimental to the Amazonian manatee population because females produce one calf every 2-3 years (Best 1982, Marmontel 2008).

The recent development related to oil exploration and extraction in the Ecuadorian Amazon poses additional risks to the manatee population. The oil era in Ecuador began in 1967 when the Texaco Gulf Consortium discovered vast amounts of crude oil in the Northern Succumbios province. In 1972, the 500 km Sistema Oleoducto Trans-Ecuatoriano [SOTE] was constructed from Lago Agrio over the Andes to Balao; it carries 324,000 barrels of oil per day. The Ecuadorian government increased expenditures for the army, air force, and modernization creating 12 billion dollars of foreign debt by 1990 after the extraction of 1.5 million barrels of crude oil. To cope with the debt, they began using oil reserves as loan guarantees. The debt increased to 13.7 billion dollars by the year 2000 in a country with a gross national product of 14.5 billion dollars. The government forced an increase in production by building a second pipeline (the Oleoducto Crudo Pesado [OCP]) and overlaying oil blocks in wildlife protected areas such as Yasuni National Park (Aaen 2006). Pipelines, oil blocks, and protected areas are mapped in Figure 2.

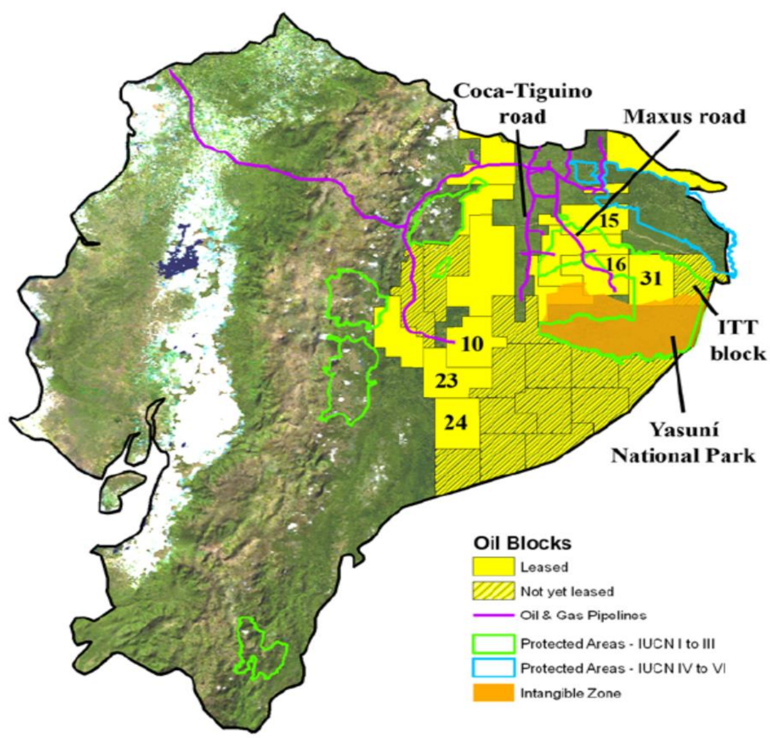


Figure 2: Focus on Ecuador. Oil and gas blocks in Ecuador, including all IUCN categorized Amazonian protected areas and key features discussed in the text. Cuyabeno Wildlife Reserve is outlined in blue and Yasuni National park is outlined in green. (Finer *et al.* 2008)

The oil extraction industry created economic problems for Ecuador, and the processes of exploration, production, and transportation have caused environmental contamination in the once pristine Amazonian rainforest (CESR 1994, Aaen 2006). CESR published a report (1994) documenting the exposure and health risk that the pollution from the oil industry has caused for humans. They analyzed thirty-three water samples for PAHs and VOCs, known toxic crude oil constituents. They found toxic PAHs in 22 samples and VOCs in 5 samples. Some chemical levels were 10 to 1000 times the legal limits set by the EPA in the United States. In 1998 an independent local laboratory surveyed 46 streams in the Eastern region and found TPH contamination in areas of oil activities, but no contamination in areas without such activities (Sebastian and Hurtig 2004). In 1999, the Instituto de Epidemiología y Salud Comunitaria, a local nongovernmental organization concerned with health issues, tested for TPH in communities near oil fields and in communities far away from the fields. In some streams, hydrocarbon concentrations exceeded the limit permitted by European Community regulation by more than 100 times (Sebastian and Hurtig 2004).

Chemical contamination endangers a vast number of species inhabiting the region. The western region of the Amazon, including parts of Bolivia, Colombia, Ecuador, Peru, and Western Brazil, is one of the most biologically diverse areas on the planet for mammals, birds, and amphibians (Figure 3) (Finer *et al.* 2008). Oil and gas development has resulted in major environmental and social impacts, including direct effects of deforestation for access roads, drilling platforms, and pipelines, and contamination from oil spills and wastewater discharges. Indirect effects arise from easy access to previously remote primary forest, causing increased logging, hunting, and deforestation and an increase in boat traffic from human settlement (Finer *et al.* 2008). These effects result in an extremely morbid outlook for all species in the Ecuadorian Amazon, and specifically, the Amazonian manatee, which is already the most endangered mammal in Ecuador (Denkinger 2010).

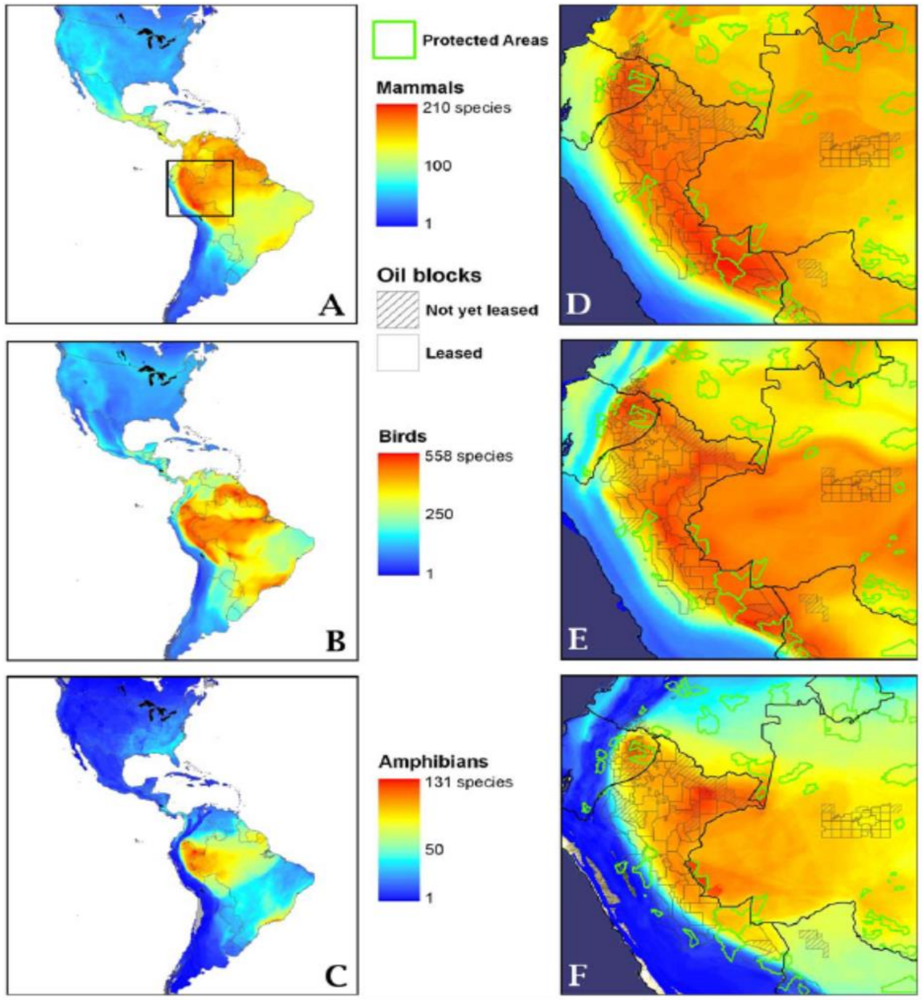


Figure 3: Overlap of oil and gas blocks with biodiversity and protected areas. The color red represents the highest concentration of species for mammals (A), birds (B), and amphibians (C) across the Americas, where the highest diversity occurs in the western Amazon. Detailed view of the western Amazon region, outlined by the box in A, for mammals (D), birds (E), and amphibians (F). In this region hydrocarbon blocks overlap areas of exceptionally high biodiversity. Protected areas shown are those considered strictly protected by the IUCN (categories I to III), yet are still being leased. (Finer *et al.* 2008)

CHAPTER II

SONAR SURVEYS AND SIDE-SCAN DATA

OBJECTIVES AND HYPOTHESES

The objectives of this investigation were to:

- Test the opposing hypotheses that the Amazonian manatee persists or has been extirpated in the Ecuadorian Amazon utilizing side-scan sonar to increase detectability.
- Determine if spatial or temporal differences in probability of manatee detection exist.
- Investigate the hypothesis that side-scan sonar is a viable and efficient method for collecting Amazonian manatee population data by comparing preliminary to final manatee observations and by comparing effort for visual versus side-scan sonar surveys.
- Test the hypothesis that manatees observed via side-scan sonar fit the Distance® detection function (decreasing detectability with increasing distance from zero line).

Not within the scope of this thesis, the long-term goal of this project was to:

- Determine if a model for assessing the trend of the total Amazonian manatee population in Ecuador could be developed; this will be addressed elsewhere.

INTRODUCTION AND BACKGROUND

Amazonian manatees are endemic to the turbid, tannin-rich, lagoons, oxbow lakes, and tributaries of the heavily vegetated Amazon River basin (Cantenhede *et al.* 2005, Marmontel 2008) making them difficult to observe in their environments. There are two methods used by researchers to detect manatees: aerial surveys and boat or land-based surveys (Gonzalez-Socoloske et. al. 2009). Both methods rely on visual detection and are not well suited for areas of low water visibility (Ackerman 1995). The narrow

winding shape of the riverine habitats and overhanging vegetation increases the challenge (Gonzalez-Socoloske et. al. 2009, Timm et a. 1986).

Aquatic mammals are inherently difficult to observe, and the Amazonian manatee is a very secretive creature that spends most of its time submerged as an adaptation to hunting pressures (Marmontel 2008). It is cryptic, inaccessible, shy, and secretive (Timm *et al.* 1986, Rosas 1994). They breathe every 3-5 minutes and can stay submerged up to 14-25 minutes (Denkinger 2010, Husar 1977). An Amazonian manatee demonstrates its secretive nature by simply raising its nostrils above the surface, respiring and sinking vertically, leaving a scarcely perceptible ripple (Reeves et. al. 1996). Amazonian manatees could easily be undetectable, hidden amongst patches of floating plants (Colares and Colares 2002).

Valid population estimates for the species are unknown (Marmontel 2008). Due to the elusive nature and low water visibility, Amazonian manatees are difficult to observe in the wild. Population studies are rare, data are sparse, and current local population estimates are unsubstantiated (Timm *et al.* 1986, Rosas 1994, Denkinger 2010). In the 1970's, the number of Amazonian manatees inhabiting the entire Amazon basin was estimated to be 10,000 and declining due to persistent hunting (Husar 1977). However, these numbers must be regarded with caution since they are supported by very little empirical data (Marmontel 2008).

There is no information describing the proportion of that estimate inhabiting Ecuador in the 1970s. The "Libro Rojo de los Mamiferos Del Ecuador" [The Red Book of Mammals of Ecuador] published by several conservation groups (Tirira 2011) listed the Amazonian manatee in Ecuador as "Critically Endangered". This classification includes the following criteria among others: the population size has been reduced by more than 80% within 10 years or 3 generations and the number of mature individuals is less than 250 (Tirira 2011).

Manatees were reported to be abundant in the early 1980s in most of the lagoons and rivers of Cuyabeno Wildlife Reserve in Ecuador, but were persecuted for meat by Peruvian and Ecuadorian militaries (Marmontel 2008). In 1983, the presence of

Amazonian manatees in Rio Aguarico and Rio Cuyabeno of Ecuador was investigated based on second hand reports of its occurrence (Timm *et al.* 1986). Approximately 10 individuals were observed and first-hand sighting accounts by locals in Rio Cuyabeno, Laguna Grande, Laguna Zancudo Cocha, Lagartococha, Rio Yasuni, Rio Anangu, Rio Samiria, San Francisco, Laguna Imuya, and Loro Cocha in Peru were reported. Manatees were abundant in Lagartococha and Siona Indians had harvested the manatee for generations (Timm *et al.* 1986).

Although this was the first report on the presence of the Amazonian manatee in Ecuador, if the observed take levels were to continue the species would be eradicated from Ecuadorian waters within 10-15 years (Timm *et al.* 1986). The Siona Indians then practiced a self-imposed ban on hunting because of low manatee population numbers (Timm *et al.* 1986). However, some of the Siona never knew about this ban, and it seems possible that hunting continued (Marmontel 2008). Amazonian manatees still exist in the Cuyabeno River, but likely in low numbers and reportedly, have not been seen since 10 years ago by Peruvian hunters in the Lagartococha system (Marmontel 2008). There is contradictory information from recent interviews but the general consensus is that the population is declining (Marmontel 2008).

A second population study of Amazonian manatees in Ecuador was conducted from 1996-1999 in Rio Cuyabeno and Lagartococha (Denkinger 2010). The study used visual survey methods and only reported 4 sightings (3 in Cuyabeno and 1 in Lagartococha) in 454.05 observation hours covering 201 km. Forty to forty-nine incidental manatee sightings were reported in Laguna Grande and Cuyabeno for the years of 1996-1998 by park rangers, natives, tour guides, and the observer (Denkinger 2010). The once abundant population in Lagartococha had dropped drastically and hunting was far from sustainable (Denkinger 2010).

Aerial surveys are commonly used to document the distribution and relative abundance of the Florida manatee (*Trichechus manatus latirostris*) (Ackerman 1995). They are conducted in the winter months at known aggregation sites and the results are used to assess population trends (Ackerman 1995). The focus of current research is to improve estimates of population size and trend by addressing several inadequacies

(Ackerman 1995, Lefebvre *et al.* 1995). Aerial-survey based estimates of manatee abundance are biased because of visibility and sampling problems (Lefebvre *et al.* 1995). These include perception bias, availability bias, absence bias, and environmental factors (Lefebvre *et al.* 1995). Aerial surveys are not well suited for turbid, murky waters, winding rivers, or over-hanging vegetation (Ackerman 1995, Gonzalez-Socoloske *et al.* 2009, Timm *et al*. 1986), and Amazonian manatees do not congregate like Florida manatees in well-known, easily observed areas.

The same problems associated with complex environments such as the Amazon Basin are encountered in boat or land-based surveys (Denkinger 2010). They are comparatively inexpensive, but cover small spatial scales, are very labor intensive, and have very low detection rates, especially in areas where manatees are hunted (Gonzalez-Socoloske *et al.* 2009, Denkinger 2010). Although these techniques have proven successful for monitoring the Florida manatee, they are not feasible in complex habitats, due either to constraints associated with habitat or to the high costs involved both monetarily and temporally (Gonzalez-Socoloske *et al.* 2009). The only areas that have been reliably surveyed are those with primarily clear, coastal marine water, or where obligatory seasonal clustering occurs due to the inability of manatees to tolerate low temperatures (Gonzalez-Socoloske *et al.* 2009). The seasonal clustering of Amazonian manatees during low water may provide a good opportunity for surveys, however, preferred deep water lagoons in Ecuador have yet to be identified.

Due to the difficulties associated with observing manatees and obtaining reliable counts in complex, freshwater habitats, the use of sonar systems to detect manatees has been the subject of recent research. In the 1980s, several attempts were made to detect manatees using sonar acoustic technologies with the primary focus to prevent manatee deaths by floodgates, canal locks, and boat collisions (Gonzalez-Socoloske *et al.* 2012, Bowles *et al.* 2004). Based on target strength measurements (the proportion of sound that is reflected by a target back to the source) of other large marine mammals, good sonar returns were expected from manatees at ping frequencies of 10 and 80 kHz (Au 1996, Bertrand *et al.* 1999 as cited by Bowles *et al.* 2004). The attempts were limited in scope and produced inconclusive results. Some of the studies reported good sonar returns and

detections, but others reported surface and bottom scatter, sonar shadowing, high background noise levels, vessel-generated turbulence, and low-amplitude returns as reasons for limited success (Bowles *et al.* 2004).

Past studies of various other marine mammals reported measurements of good target strength (Gonzalez-Socoloske and Olivera-Gomez 2012). Dolphin target strength is best near the lungs between the dorsal and pectoral fins (Au 1996 as cited by Gonzalez-Socoloske and Olivera-Gomez 2012). Based on the good target strengths measured for other marine mammals and the fact that manatees have elongated lungs that are positioned dorsally along the long axis of the body (Rommel and Reynolds 2000), the hypothesis that strong acoustic returns in manatees could be measured was still valid (Gonzalez-Socoloske and Olivera-Gomez 2012).

In 2005, Gonzalez-Socoloske *et al.*(2009) tested a high frequency (262-455 kHz) side-scan sonar unit developed by Humminbird® (Model 987c SI, Johnson Outdoors Inc., St. Racine WI, USA; see Table 1) in three locations ranging from clear water in Florida to dark tannin-stained water in Honduras and Mexico (Gonzalez-Socoloske *et al.* 2009, Gonzalez-Socoloske and Olivera-Gomez 2012). Their goals were: (1) to develop a technique that could reliably detect manatees in locations where they are difficult to see through turbid, tannin-stained water; and (2) to observe manatees over a large area without the necessity of the animal crossing the beam (Gonzalez-Socoloske *et al.* 2009). All previous efforts to detect manatees with sonar had used stationary echo-sounder systems (Dickerson *et al.* 1996, Jaffe *et al.* 2007 as cited in Gonzalez-Socoloske *et al.* 2009). Some scanning systems were tested (rotating 360°); however, they work under the same principle of measuring a change in reflectivity against a constant background. Side-scan sonar systems create an image of the surveyed area's acoustic signal as it moves in a linear direction (Gonzalez-Socoloske *et al.* 2009).

Table 1: Technical Specifications of Humminbird Units Equipped with Side-Scan Sonar (Gonzalez-Socoloske and Olivera-Gomez 2012)

Humminbird® Fishfinder Model				
	981c SI*	987c SI*	797c2 SI*‡ 798c SI* 798ci HD SI	898c SI 997c SI* 998c SI 1197c SI* 1198c SI
Side-Scan Sonar				
Beam frequency and angle	262 kHz (2) 84° at -10 dB	262 kHz (2) 84° at -10 dB 455 kHz (2) 40° at -10 dB	455 kHz (2) 86° at -10 dB	455 kHz (2) 86° at -10 dB 800 kHz (2) 55° at -10 dB
Total coverage†	180°	180°	180°	180°
Max depth (m)	33.3	33.3	50	50
Lateral range (m)	80	80	120	120
Echo Sounder Sonar				
Beam frequency and angle	50 kHz 74° at -10 dB 200 kHz 20° at -10 dB	50 kHz 74° at -10 dB 200 kHz 20° at -10 dB	83 kHz 60° at -10 dB 200 kHz 20° at -10 dB	83 kHz 60° at -10 dB 200 kHz 20° at -10 dB
Max depth (m)	762	762	457	457

*Legacy Models
†Coverages reported by the manufacturer (Installation and Operations Manual for: 981c SI & 987c SI; 997c SI; 898c SI & 998c SI; 1197c SI; and 797c2 SI. Available from URL http://www.humminbird.com/support/ProductManuals.aspx)
‡Model used for this thesis

Sonar stands for Sound and Navigation Ranging and involves emitting specific frequencies of acoustic beams into a matrix, such as a body of water, and measuring the return signal. In the Humminbird® 797c2 side-scan sonar system, distance is determined by measuring the time between the transmission and reflection of a sound wave off of an object; it then uses the reflected signal to interpret location, size, and composition of an object. The sound pulses "echo" back from objects in the water and are displayed on the LCD screen. Each time a new echo is received, the old echoes are moved across the screen, creating a scrolling effect. Sonar travels from the surface to a depth of 240 ft (70 m) and back again in less than ¼ of a second (Humminbird® 797 User Manual 2006).

Side-scan sonar systems function by emitting a fan shaped pulse at a wide angle perpendicular to the movement of the sensor (see Figure 8). The sonar unit is either mounted directly onto the vessel or towed in a capsule (Gonzalez-Socolske and Olivera-Gomez 2012). Side-scan sonar has been used for a variety of applications that utilize the instrument's underwater imaging clarity and range. Some of the applications include underwater mapping of bottom topography and seafloors (Dura 2004), classification of bottom types (Barnhardt 1998), and characterization of resting holes for the Antillean manatee (*Trichechus manatus manatus)* (Bacchus 2007). Side-scan sonar has also been used in archeological applications and to infer animal behavior from benthic features such as sediment scars (Gonzalez-Socolsoke and Olivera-Gomez 2012).

A variety of manatee habitats and environmental conditions have been tested using side-scan sonar by conducting target surveys where manatees could be counted visually in order to determine its usefulness in studying wild manatees and estimate a preliminary detection rate for the sonar unit (Gonzalez-Socoloske et al. 2009). Water clarity, time of day, and other environmental factors had little effect on the quality of the sonar images produced, with the exception of surface water movement (Gonzalez-Socoloske et al. 2009). The Florida clear water trials, at times, produced images which were not as clear as the Honduras and Mexico tannin-stained, turbid water trials. This may be explained by the lack of a strong current in the Mexican and Honduran waters compared to Floridian waters. In addition, heavy boat traffic and high winds in Florida's Crystal River may have distorted the images (Gonzalez-Socoloske et al. 2009). Preliminary detection rates for manatees using the side-scan sonar were 81-93%, the sonar produced no noticeable behavioral response in the manatees, and the sonar frequency was well above the known hearing range for manatees (6 to 20 kHz, Gerstein et al. 1999) at >200 kHz (see Table 1). Table 2 summarizes the conclusions from preliminary testing of the side-scan sonar method for detecting manatees (Gonzalez-Socoloske et al. 2009, Gonzalez-Socoloske and Olivera-Gomez 2012).

Table 2: Summary of Advantages and Limitations of Using Side-Scan Sonar for Manatee Research (Gonzalez-Socoloske and Olivera-Gomez 2012)

Advantages	Limitations
Humminbird® Sonar Systems	
Compact units, with built-in screens	Weak cables can break after repeated use
Additional data (see Figure 9)	Screen size and image resolution
Affordable, can be shared between groups	Glare on screen during sunny days
Records screen captures and scans	
Transom-mounted transducer	
As a technique for manatee surveys	
High detection rate (>80%)	Detection range of <20 m (40 m swath)
Greatly reduces availability bias	Limited to line surveys at constant speeds
Allows for night surveys	Limited to perpendicular detection
	Small spatial scale vs. aerial surveys
	Possible false positives and false negatives
Manatee behavior	
Sedentary lifestyle	Manatees moving out of detection range

STUDY AREAS

This investigation was conducted in three expeditions to eastern Ecuador and the border near Peru which explored northern Yasuni National Park, eastern Cuyabeno Wildlife Reserve near Lagartococha, and western Cuyabeno Wildlife Reserve near Lago Agrio in March, May, and July 2011. The study regions are part of the Amazonian river system which extensively innervates South America. Yasuni National Park and Cuyabeno Wildlife Reserve are located north and south of the large Napo River (Rio Napo), a tributary of the Amazon River, in the Orellana and Sucumbios provinces of Ecuador (See Figure 4).

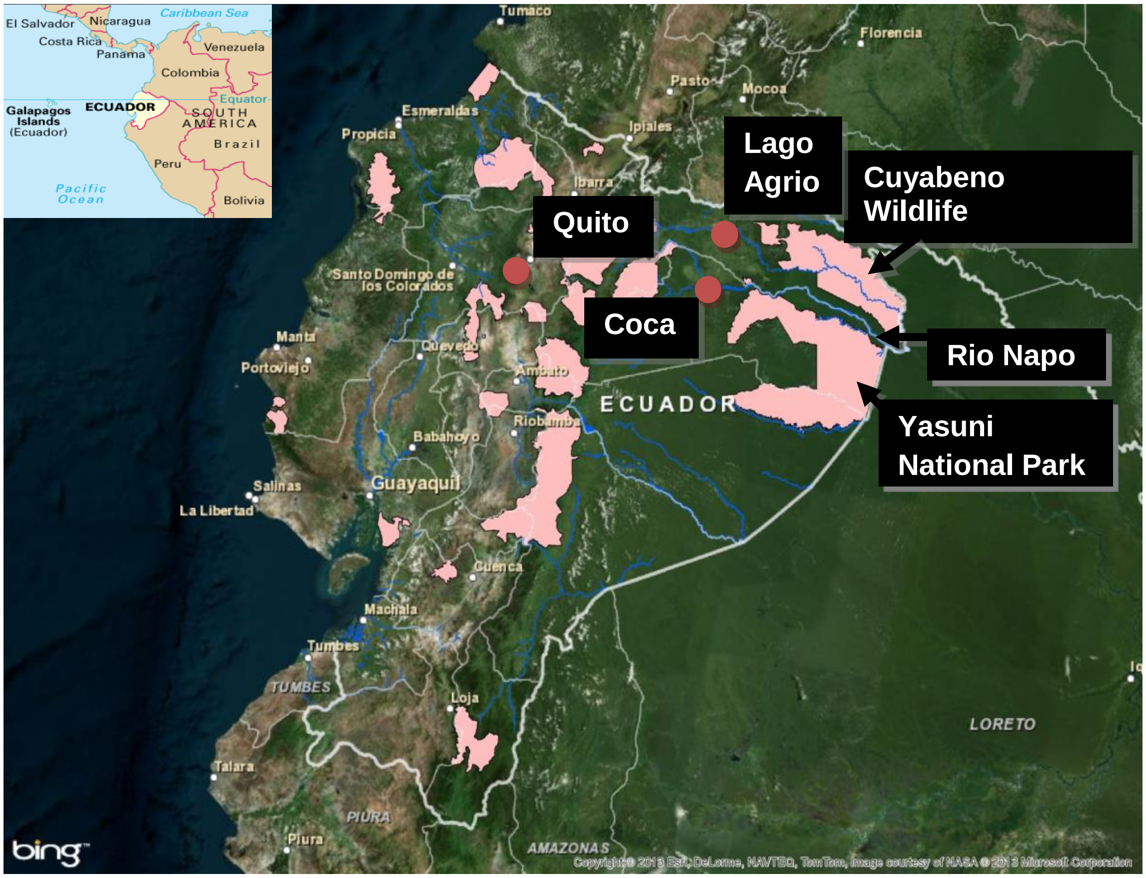


Figure 4: Map of Ecuador showing Quito, Coca, Lago Agrio, and the investigated protected areas, Cuyabeno Wildlife Reserve and Yasuni National Park, in the Amazon rainforest near the Napo River. This map was created using ArcMap.

Expeditions 1 and 2 began in Coca, Ecuador (Puerto Francisco de Orellana) on the Rio Napo after a short flight from Quito. A motorized boat (Macao) provided by WCS was used to travel upstream to Lakes Añangucocha and Yuturi located on tributaries of the Rio Napo about halfway between Coca and the Peruvian border in March 2011. During this expedition, the lagoons Tambucocha, Jatuncocha, Yuturi, Huiririma, Cadiyuturi, and Anangu were surveyed (See Figures 4 and 5).

The second expedition in May 2011 was conducted from the same starting point in Coca, however, instead of staying along the Rio Napo, the Macao traveled further east to the Peruvian border up Rio Lagartococha. The lagoons Garcacocha, Piuri, Urcococha, Yarinacocha, Redondococha, Lagartococha, Clavococha, Huyracocha, Imucocha, Imuya,

Delphincocha, Bocana de Renaco, Zunicocha, and Patococha were surveyed. After a few days surveying the Lagartococha area, the observers traveled back west of the Peru/Ecuador border to Rio Cocaya. Rio Cocaya and the lagoon Caballococha were also surveyed (See Figures 4 and 6).

A third expedition was conducted in July 2011, beginning on the Cuyabeno river two hours south-east by road from Lago Agrio. The Cuyabeno River was descended to the Cuyabeno Wildlife Reserve. While investigating this area, the lagoons Ancacocha, Canangueno, Cocodrilococha, Macurococha, Manzacocha, Lorococha, Patococha, Cuyabeno, Charapacocha, Mateococha, and a segment of the Rio Cuyabeno were surveyed for manatee population and habitat data (See Figures 4 and 7).

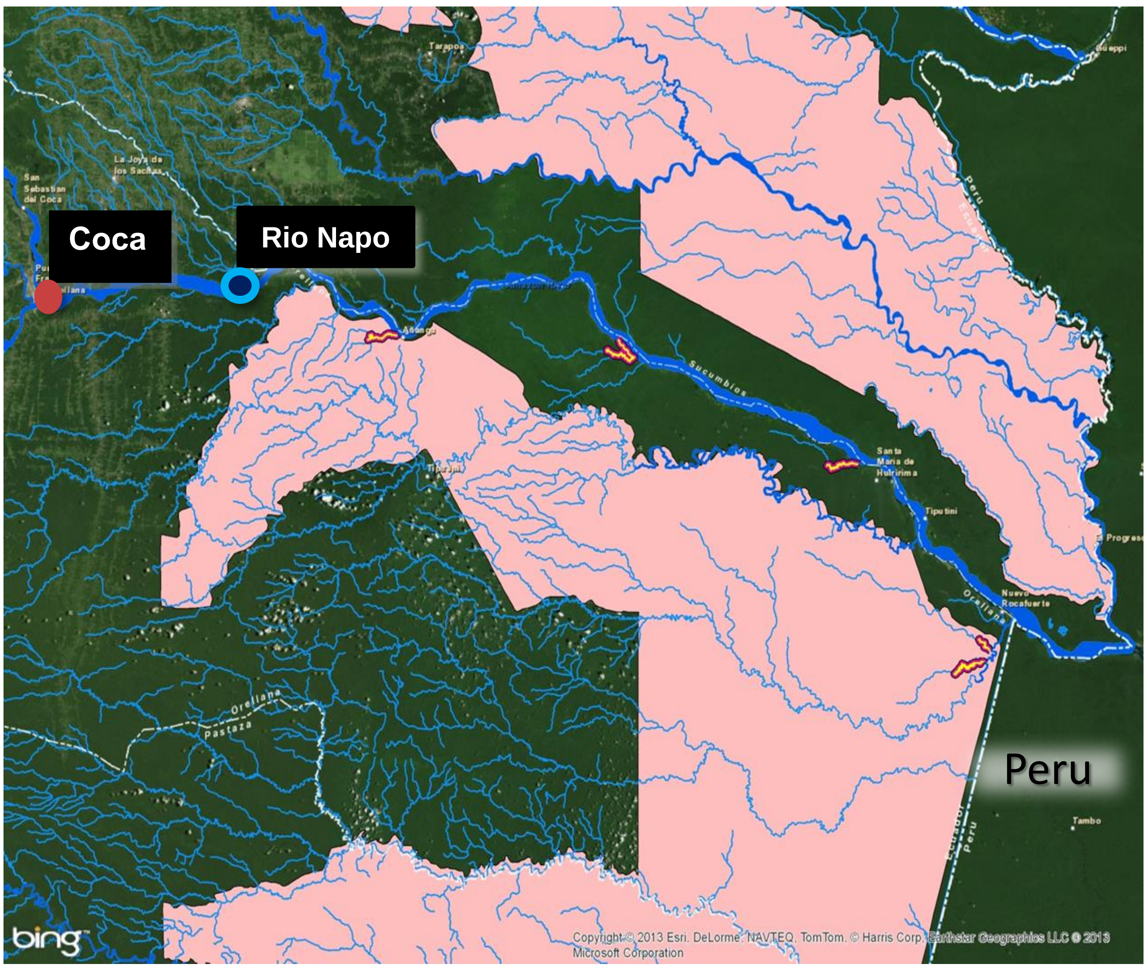


Figure 5: Map showing surveyed areas in yellow in the Orellana province in Yasuni National Park from the March 2011 study. Rio Napo was descended from Coca to Yasuni. This map was created using Arcmap

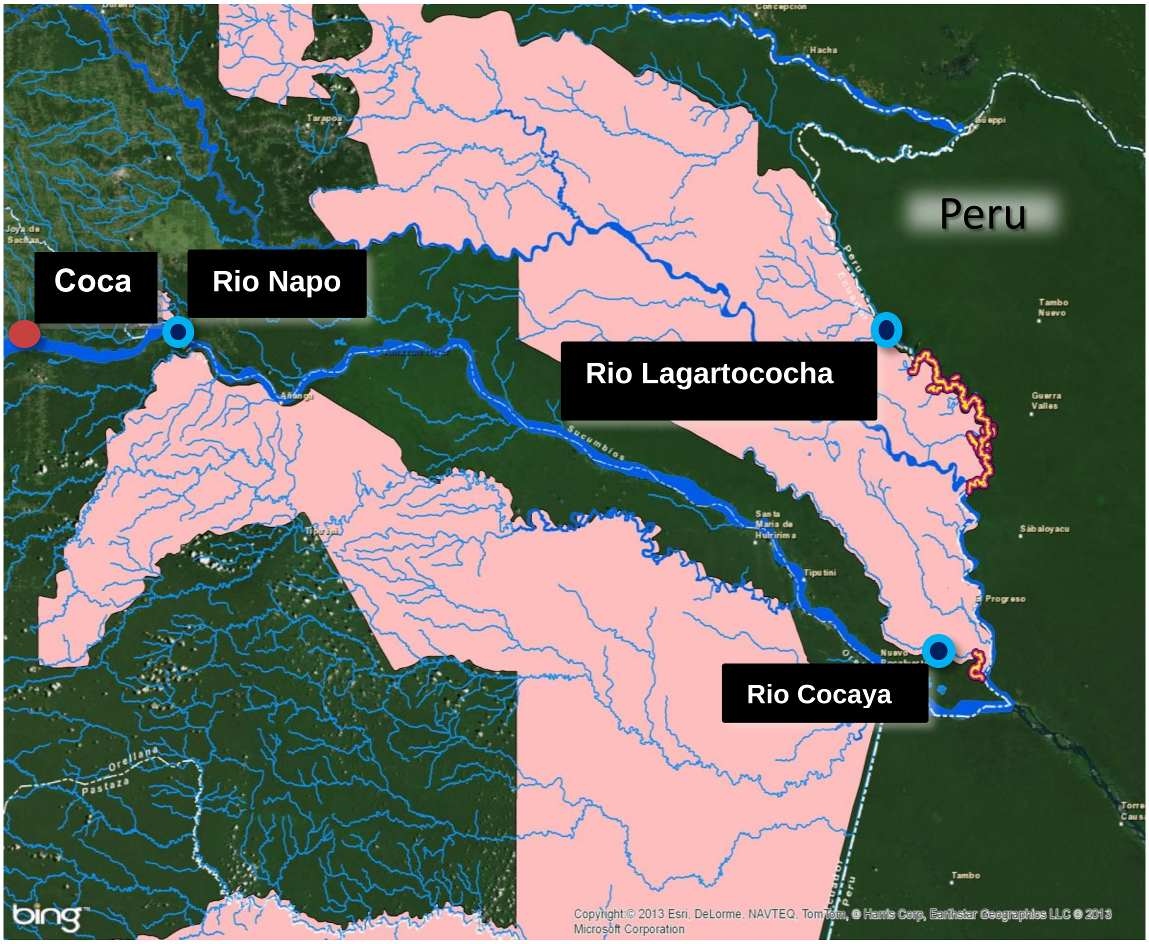


Figure 6: Map showing surveyed areas in yellow in the Sucumbios province near the Peruvian border in Lagartococha and Cocaya from the May 2011 study. Rio Napo was descended from Coca to Lagartococha and Cocaya. This map was created using Arcmap

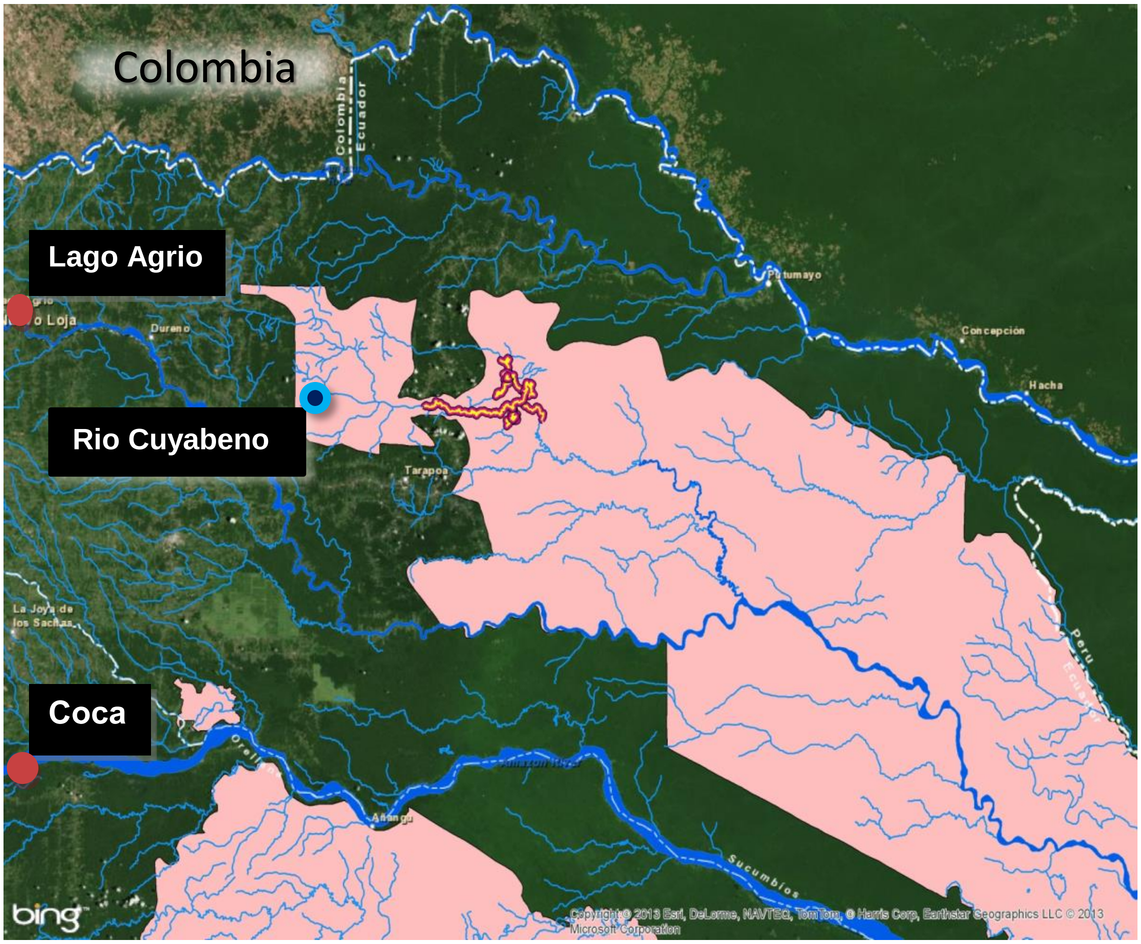


Figure 7: Map showing surveyed areas in yellow in the Sucumbios province in Cuyabeno Wildlife Reserve from the July 2011 study. We traveled two hours southeast by road from Lago Agrio to Rio Cuyabeno. This map was created using Arcmap

METHODS AND DESIGN

SIDE-SCAN SONAR AND FUNCTIONALITY

Side-scan sonar systems function by emitting a fan shaped pulse at a wide angle perpendicular to the movement of the sensor (see Figure 8). The Humminbird® 797c2 sonar system is equipped with a dual beam vertical depth finder that emits sounds at a frequency of 200 kHz at a 20° angle and intensity of -10db and at a frequency of 83 kHz at a 60° angle and intensity of -10db. The lateral beam for side-imaging emits sounds at a 455 kHz frequency at an 86° angle from vertical at an intensity of -10db (See Table 1, Gonzalez-Socoloske and Olivera-Gomez, 2012). The two center beams are downward facing echo sounders and the side beam is positioned at a different wider angle. This 455 kHz frequency beam offers a total reported coverage of 180° (See Table 1, 797c2 GPS Chartplotter Operations Manual).

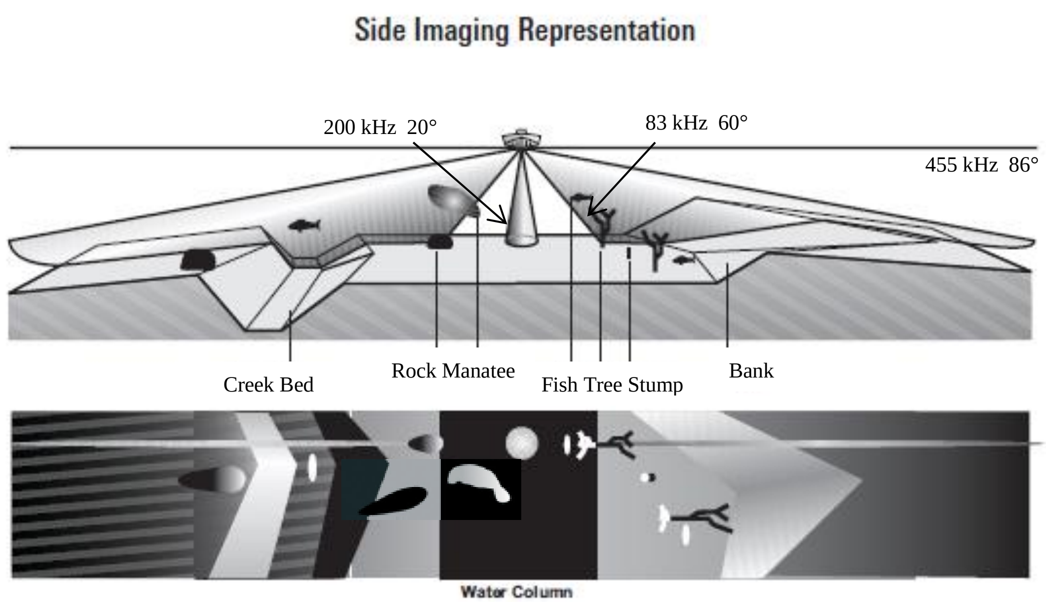


Figure 8: A diagram of the use of vessel-mounted side-scan sonar in the field. The top image shows a vessel equipped with a sonar unit. The Humminbird® 797c2 acoustic beams are depicted at approximate angles and labeled with corresponding frequencies. The top figure depicts several identified objects which may be encountered and produce acoustic returns during field surveys. The bottom image demonstrates the acoustic return for each object and how it appears on the unit's screen. Source: 797c2 GPS Chartplotter Operations Manual; Modified using Gonzalez-Socoloske *et al* 2009.

The side-scan sonar unit is mounted directly to the boat and produces an image of what is present below the surface of the water. Humminbird® 797c2 side-scan sonars come equipped with a 12.5 cm digital screen read-out, which assembles images as they are produced. Therefore, no computer or external software is required for surveys. The images are created from a series of cross transect slices which are captured at a user defined frequency from all three sonar beams. The sonar unit is equipped with a built-in global positioning system (GPS) receiver for latitude, longitude, and time, and the sonar transducer is equipped with a thermometer for surface water temperature. Screen captures and entire recordings of sonar surveys can be saved. Along with the sonar images, the recordings will retain boat speed, geographic coordinates, surface water temperature, date, time of day, and water depth. Units are powered by 12 V batteries and have a power draw of 615-1300 mA depending on the model (Gonzalez-Socoloske and Olivera-Gomez 2012).

IMAGE INTERPRETATION

The digital read out of the Humminbird® unit consists of a single image where the top is the most recent sonar cross transect slice and the slices get “older” as you move down the image. Complete refresh of the screen occurred approximately every 10 seconds (Humminbird® chart speed setting of 5). Collectively, these slices form an image of the state of a body of water including bottom topography and objects in it at the moment the sonar transducer passes over them. Each image can be thought of as a “snapshot in time”. As the vessel moves in a straight line new acoustic data are pushed down in a top to bottom conveyor belt fashion (Gonzalez-Socoloske and Olivera-Gomez 2012). The two narrow blue lines in the center represent the mid-point of the sonar recording and also correspond to the trajectory of the vessel situated at the top (See Figure 9). Side-scan sonar images consist of a right and left side divided by a lighter or darker section in the middle (depending on the user’s contrast and color settings). This middle section represents the water column directly beneath the boat and is formed by the echo sounder acoustic beams. The rest of the image is interpreted as the “bottom surface return” formed by the 455 kHz wide angle acoustic beam starting below the boat and

continuing laterally away from the boat until the edge of the user defined lateral range up to 120 m (per side).

Objects in the water appear black and cast a white shadow on the bottom when using the black and white color contrast (BWCC) setting observed in Figure 9. Objects directly below the boat appear in the echo sounder return near the boat trajectory. Objects which were situated further from the boat laterally were observed in the bottom surface return. Using the BWCC (a negative of the default), objects and surface features appear on the acoustic return in different shades ranging from black (high target strength) to white (low target strength) depending on the reflectivity of the material. The darker the object is on the image, the greater the target strength and reflectivity. Shadows appear white on the BWCC because they have no target strength or reflectivity and represent the section of acoustic response blocked by an object.

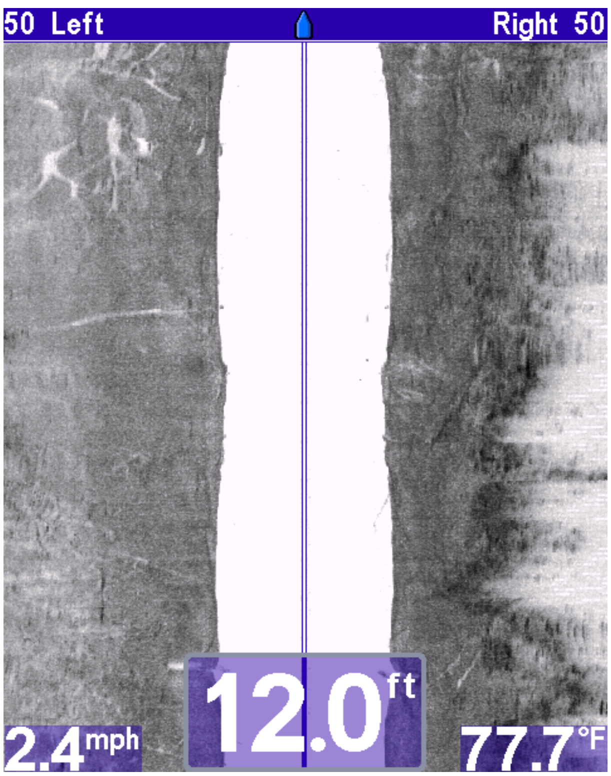


Figure 9: Digital readout from the Humminbird® 797c2 side-scan sonar unit showing the echo sounder return, bottom surface return, depth, water temperature, speed of vessel, and lateral range.

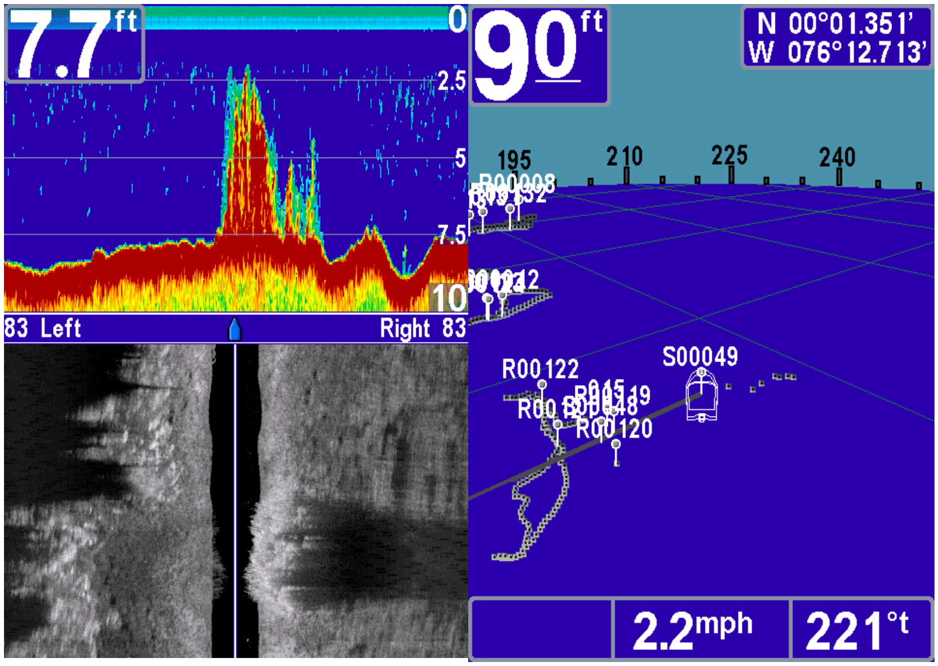


Figure 10: Screenshots of the digital screen display of the Humminbird unit demonstrating different views, options, and information.

The screen display can be toggled between left and right views or display both sides of the side-scan sonar response simultaneously (Gonazalez-Socoloske and Olivera-Gomez 2012). Half of the digital screen can be used to display the echo sounder response below the boat or the left or right side-scan response. There is a view that lists recordings and snapshots on the currently installed SD card. The unit is equipped with chart and map views which display a map with recording tracks of the vessel and the current position. A chart or map of the study area can be uploaded to the Humminbird®, however, that function was not used for this project. (See Figure 10). The user can display boat trajectory, surface temperature, speed, latitude and longitude, depth, and time, or change the color contrast settings, increase or decrease the lateral range, and change the image capturing frequency of the sonar beams.

The ideal boat speed for obtaining the best acoustic images is between 2.5 and 7.0 km per hour (Gonzalez-Socoloske and Olivera-Gomez 2012). Vessel speed and swimming speed of a target can alter the relative size of objects detected by the side-scan-sonar. Depth distortions can also present themselves in the side-scan sonar acoustic response images. The water column can take up a disproportionate amount of the sonar

image depending on the depth (See Figure 29). As an example, if two screen captures are taken at different water depths (1 m and 5 m) with the same lateral range (10 m), the first will have 9/10 of the image for side-scan response whereas the second will have 5/10 of the "image space" to fit the same benthic response. This is because the echo sounder response increases in width proportionately with increasing depth. (Gonzalez-Socoloske and Olivera-Gomez 2012).

Bottom topography is evident from the shadows and acoustic reflection gradient. Shadows (created by objects blocking the acoustic beam) are used to determine shape and form of objects and prove useful for helping to identify and interpret the acoustic reflection. It can be difficult to interpret side-scan sonar images without valuable field experience to orient an observer. It is essential to be able to observe an object in the environment and then observe the acoustic response recorded. Otherwise, there are things which could easily be mistaken for manatees by an untrained observer (see Figure 11).

Objects could be interpreted as manatees by an untrained observer when they are not. In Figure 11, there are six images demonstrating the acoustic responses of different types of objects. Figure 11A is a left and right view of side-scan sonar beams on either side of the boat. The light blue streaks near the centerline are paddle strokes producing a response. Figure 11B demonstrates how tree roots appear on the left and right view of side-scan sonar beams. The outlines of the roots and branches are visible due to the shadows created. Figure 11C shows a tree stump on the left benthic return. Tree stumps can have a similar girth to manatees and an equally strong acoustic response. It takes a trained eye and field experience to interpret the shape and shadow correctly. Figure 11D contains heavily vegetated areas on the left bottom return and a lot of debris along the right. Figure 11E is an example of how the sonar image appears when the canoe is not moving or swaying slightly from side to side with the current. The transducer keeps capturing the same slice of river bed and dark and light streaks are formed. Finally, Figure 11F demonstrates the acoustic response of standing aquatic trees and the long shadow they form. Any of these objects could be mistaken for a manatee to an untrained eye and exemplify the importance of detailed interpretation. Manatees produce a signature shadow which trees, fish, rocks, and branches do not (See Figures 12 and 13).

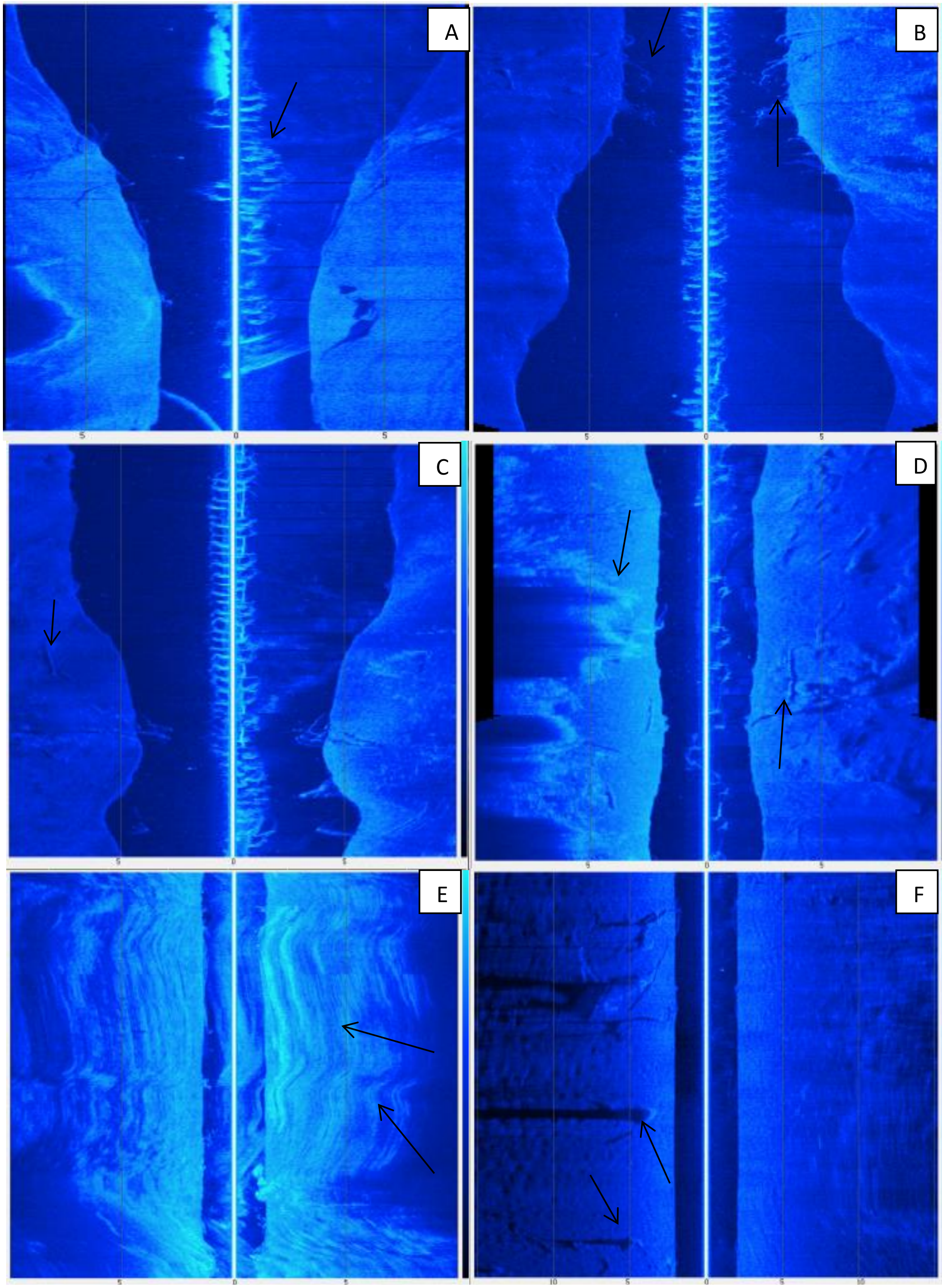


Figure 11: Lateral view screen captures from sonar surveys in Ecuador demonstrating the acoustic responses of items which could be mistaken for manatees to an untrained observer. The acoustic responses of (A) paddle strokes along the center line, (B) tree roots and branches on upper left and right sides, (C) a tree stump on the center left side of the lateral view, (D) vegetation on the left and benthic debris on the right, (E) unmoving vessel or swaying side to side, and (F) standing aquatic trees. All produce a response different from that of a manatee.

In addition to trees, branches, fish, and other objects in the river, manatees are detected as well as the shadow produced because the animals absorb or deflect the sonar beams. The criteria for determining if an object is a manatee is the signature unique peanut shape, morphology of a manatee: paddle shape of the tail, small head, and flippers, and the signature shadow (see Figures 12 and 13). The exact length of a manatee cannot be determined because it is influenced by vessel speed and water depth distortions. However, the approximate length of a manatee's acoustic response compared to other objects and the lateral range scale can be used as an indicator. A large shadow caused by the presence of a manatee appears on the acoustic image and indicates a "blocked signal". It is perceived as a lengthy dark or light "manatee" shape (depending on the color scheme employed by the observer). It indicates a large animal was in the water column absorbing all of the acoustic beams, blocking the transmittance of those beams, and preventing the imaging of other objects past the animal. The size and shape of this shadow is influenced by the orientation of the manatee and distance from the sonar transponder (See Figure 13), but it is always produced. Manatee calves appear smaller than adults.

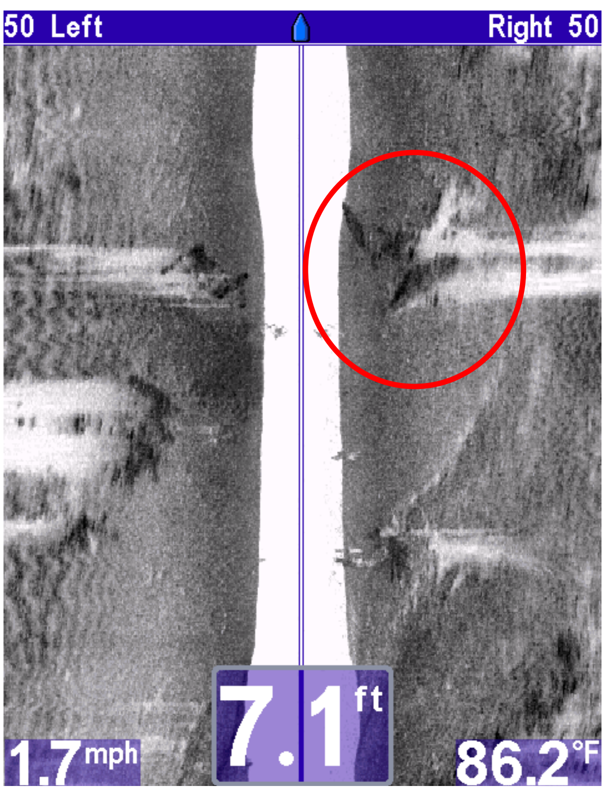


Figure 12: Screen capture from this study which demonstrates a manatee calf/cow pair acoustic signal with signature peanut shapes and shadows.

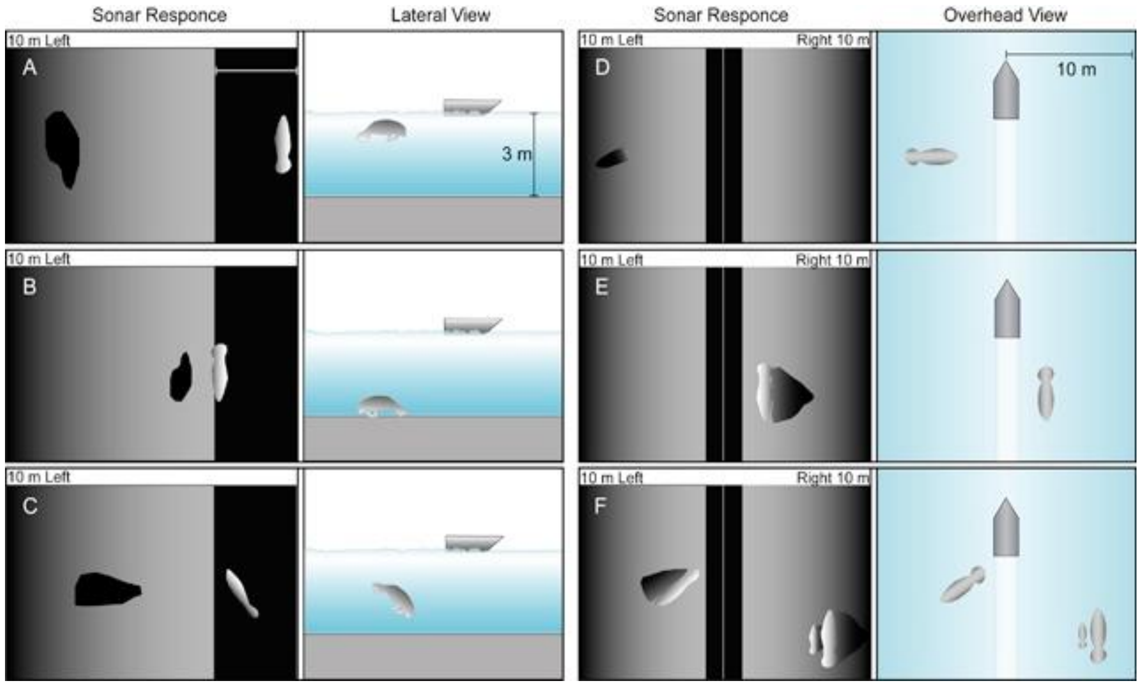


Figure 13: Model of acoustic images of manatees produced by side-scan sonar according to body position and manatee location relative to the boat from Gonzalez-Socoloske and Olivera-Gomez 2012.

SAMPLING DESIGN

A bow-mounted Humminbird® 797c2 side-scan sonar system was used to detect Amazonian manatees following the protocols described in Gonzalez-Socoloske *et al.* (2009) and Gonzalez-Socoloske and Olivera-Gomez (2012). Four aspects of the protocol were modified. The boat was propelled by paddling instead of a motor to prevent manatee avoidance behavior. The boat speed and the trajectory were haphazard rather than pre-designed. Ihe two observers were inexperienced with side-scan field interpretation. Observer experience could have been increased with preliminary control trial surveys, however, there was no funding or time allotted. Finally, the Humminbird® transducer was mounted to the bow and it is designed to be transom mounted.

Manatee population data were obtained by recording and capturing images from side-scan sonar surveys in rivers and lagoons in the study areas (See Figures 4-7). GPS data were also recorded on a Garmin GPS device for all canoe surveys (See Appendix I). Recordings were obtained within areas of the wildlife reserves, which were secluded from human activity and were pre-determined by Dr. Edward O. Keith with the

assistance of Galo Zapata Rios, M.Sc. and Victor Utreras of the WCSE (Keith 2010). The areas that were transected during surveys were also selected due to proximity to ranger stations. It was important to be able to travel to the survey sites, take recordings, and then return to the camp in one day. According to Galo and Utreras, surveying at night was not advisable due to the danger of decreased visibility hindering navigation on the river. In addition, it would be harder, if not impossible, to ground truth manatee sonar detections at night.

Our surveys began with a 2-4 hour journey at 0700 hours in a WCSE provided motorboat containing a canoe, paddles, and supplies for the day. Once the study site was reached, the canoe was unloaded and three observers were positioned in the forward, middle, and aft positions of the canoe. The Humminbird® 797c2 side-scan sonar system has a transducer, a digital screen, a GPS transmitter, and a power supply. The sonar transducer was mounted to a 45 cm wooden plank. This plank was attached to the front of the canoe such that the sonar transducer was submerged. Next, it was connected to the digital screen and GPS receiver which were attached to a 90 x 30 cm wooden board. The board was placed upon the middle observer's lap, and the unit was easily controlled from there. Finally, a 12V battery was placed in the bottom of the canoe. Red and black electrical cables were attached to the digital screen and the 12V battery.

After the Humminbird® was set-up and the observers were in position, the forward and aft observers would paddle and steer the canoe to different rivers and lagoons in the selected study sites (see Figures 4-7). Speed was maintained between 3.2 and 6.4 km/hr. Observers visually ground-truthed manatee sightings as indicated by the sonar. The criteria for confirming the sighting as an Amazonian manatee were 1) observing the characteristic rounded snout with two nostrils, black skin, air bubbles, and ripples during respiration behavior, 2) observing the head, black and white markings, snout, and prehensile lips during surfacing behavior (eating, mating, breaching) or 3) observing the peanut shaped body and paddle shape of the tail from the boat.

The observer controlling the digital screen would power the unit on once the equipment was set in place. The unit would record the survey if the observer pressed the record option on the digital menu. For this survey, the side-scan sonar data were recorded

opportunistically in the study sites. In essence, haphazard, non-overlapping snippets of the study areas were recorded based on the observer's choice and limitations such as battery life, algae, dry spots, or available memory. Recordings were saved as .son files to four 1GB Sandisc (SD) cards and assigned a unique number sequentially each time data collection started and stopped. Each of the three expeditions had unique SD cards for recordings, which ranged from 5 minutes to 120 minutes depending on the size of the assessable area and battery power. The digital screen constantly showed the sonar image when powered on even when not recording. Snapshots were taken using the MARK button, which caused the unit to capture a screenshot of the current sonar view in .bmp format and save it to the SD card associated with a unique number. The snapshots were taken when possible manatee detections were noted by the observer controlling the sonar equipment, both when recording and when not recording. In addition, GPS data for the entirety of canoe surveys were recorded on a Garmin device from WCSE by Rios and Utrera.

DATA ANALYSIS

The physical images and recordings from the side-scan sonar surveys were analyzed further in the lab to assess false negatives and confirm preliminary detections. Field observer errors are presented in results. The possibility for missed visual manatee confirmations existed due to the nature of the study region. In addition, the potential for false negatives where a manatee was seen by an observer and missed by the sonar system existed. Furthermore, due to debris and vegetation in the study region, an observer could mistake an object in the water column for a manatee detection producing a false positive.

The .son recordings obtained from the sonar surveys were analyzed with the software HumViewer® (v.67 available free at http://humviewer.cm-johansen.dk/). HumViewer® allows recordings to be analyzed from a .dat file corresponding to each recording with greater detail than the digital read out of the equipment. The .dat file constructs .son and .idx files from the sonar survey into one data display. There are numerous tools for analysis in the software, and it displays the environmental data from the recording time. All recordings obtained on the three field expeditions were reviewed

in painstaking detail using the HumViewer® software. Snapshots were reviewed using Windows Photoviewer®.

Preliminary and potential manatee observations on side-scan sonar recordings and snapshots were compared to the Gonzalez-Socoloske and Olivera-Gomez (2012) model images of manatee side-scan sonar acoustic responses based on body position (See Figure 13). A different acoustic response and shadow are produced depending on the manatee's body position relative to the sonar transducer. All objects which produced acoustic responses or demonstrated manatee morphology including paddle shape of the tail, peanut body shape, small head, or flippers were assessed. If the acoustic response exhibited manatee morphology and approximate length, produced a shadow, and matched one of the manatee model images in Figure 13, it was included as a manatee observation. The inclusion criteria were conservative and provide a minimum count because they involve seeing the peanut shape, which depends on the manatee's orientation in the water column (See Figure 13). All observations were documented in a table along with the following parameters: recording or snapshot number, estimated length, perpendicular distance from boat, lateral range, latitude, longitude, date, and time. Side-scan sonar images containing the manatee observation were also saved.

Sonar survey recordings contained GPS data from each expedition. Each .dat file was converted to a .kml file containing the GPS track of all recording sessions. The .kml files were uploaded into ArcGIS and converted to .shp files creating layers in ArcMap. The data from the Garmin device were saved in .gdb file formats and were usable with the software Garmin Basecamp® (v4.1.1 available free at www.garmin.com/en-US/shop/downloads/basecamp). This software allowed the .gdb files for each of the three expeditions to be viewed and exported. The .gdb files were exported to .gpx; following this conversion, the freeware DNRGPS (v6.0.0.15 available free at www.dnr.state.mn.us/mis/gis/DNRGPS/DNRGPS.html) was used to convert the .gpx files to .shp files which were easily layered in ArcMap. The GPS coordinates for all manatee detections were uploaded to ArcMap using .csv format to create a layer. Finally, shape files for protected areas, major rivers, tributaries, oil pipelines, provinces, oil wells, and oil blocks obtained from Rios at WCSE were layered using ArcMap.

RESULTS

A total of 238.8 km were surveyed in 70 hours 49 minutes including all three study areas (see Table 3). The number of manatees observed in the field was 45. This number included 43 sonar observations in the Yasuni and Lagartococha expeditions, 0 sonar observations on the Cuyabeno expedition, and 2 visual observations on the Lagartococha expedition while not using sonar. The 2 visual detections were confirmed by local, knowledgeable guides, met the confirmation criteria, and were not recorded with sonar. The manatees breached the surface while mating; the heads were visible with 2 nostrils, and blunt, rounded snouts, prehensile lips, and black and white surface markings. None of the 43 possible sonar detections were ground-truthed according to the criteria defined in “Sampling Design”. Table 4 contains the list of preliminary manatee detections. The GPS data for these detections were taken from the Garmin® device.

Table 3 – Effort for the Three Visual and Sonar Surveys

Survey 1 (Yasuni)	Time (hh:mm:ss)	Distance km	Transect ID
Añangu	6:09:25	15.0 km	Transect 1
Huiririma	2:04:23	19.2 km	Transect 2
Jatuncocha	8:30:16	16.1 km	Transect 3
Tambococha	3:04:58	4.1 km	Transect 4
Yuturi	4:45:28	15.8 km	Transect 5
Total for Survey 1	24:34:30	70.2 km	
Survey 2 (Lagartococha and Cocaya)			
Muestreo 1	4:14:48	16.0 km	Transect 6
Muestreo 2 & 3	4:48:06	20.3 km	Transect 7
Muestreo 4a	1:15:23	2.7 km	Transect 8
Muestreo 4b	3:17:04	22.5 km	Transect 9
Muestreo 4c	0:36:54	3.1 km	Transect 10
Muestreo 5	6:35:13	15.4 km	Transect 11
Muestreo 6	2:43:59	8.1 km	Transect 12
Muestreo 7 – Cocaya	5:04:20	22.2 km	Transect 13
Total for Survey 2	28:35:47	110.3 km	
Survey 3 (Cuyabeno)			
Muestreo 1	2:57:19	8.2 km	Transect 14
Muestreo 2	3:29:21	10.6 km	Transect 15
Muestreo 3	2:42:36	7.4 km	Transect 16
Muestreo 4[a]	2:17:37	8.2 km	Transect 17
Muestreo 4b	0:43:35	3.8 km	Transect 18
Muestreo 4c	1:38:42	9.4 km	Transect 19
Muestreo 5	2:41:44	8.5 km	Transect 20
Muestreo 6	1:07:52	2.2 km	Transect 21
Total for Survey 3	17:38:46	58.3 km	
Total for Study	70:49:03	238.8 km	

Table 3: This table shows the IDs of the surveyed areas from each expedition, time surveyed, distance surveyed, a numeric identification, and total time and distance surveyed. See Appendix I for the Garmin® GPS survey tracks.

Table 4: Preliminary Manatee Observations

Expedition	Sample ID	Date	Time	South	West	Altitude
1: Yasuni	Manati 1	07-MAR-11	10:44:32	S0.99922	W75.45085	174 m
	Manati 2	07-MAR-11	13:25:33	S1.00706	W75.47566	183 m
	Manati 3	07-MAR-11	16:23:17	S0.99723	W75.46766	180 m
	Manati 4	09-MAR-11	12:36:27	S0.55073	W76.03259	221 m
	Manati 5	09-MAR-11	14:00:06	S0.54535	W76.05103	203 m
2: Lagarto Cocha	Manati 1,2	23-MAY-11	9:28:40	S0.55503	W75.22837	178 m
	Manati 3	23-MAY-11	12:59:18	S0.47636	W75.34584	185 m
	Manati 4	23-MAY-11	13:09:47	S0.47941	W75.34726	187 m
	Manati 5	23-MAY-11	13:18:26	S0.48025	W75.35024	190 m
	Manati 6,7	23-MAY-11	13:31:06	S0.47980	W75.35420	190 m
	Manati 8	23-MAY-11	14:44:55	S0.46015	W75.33676	187 m
	Manati 9	23-MAY-11	15:36:15	S0.45634	W75.32718	184 m
	Manati 10	24-MAY-11	12:10:04	S0.50543	W75.32395	193 m
	Manati 11	24-MAY-11	12:36:06	S0.50652	W75.31693	192 m
	Manati 12	24-MAY-11	13:52:32	S0.50415	W75.30745	193 m
	Manati 13	24-MAY-11	15:04:42	S0.48839	W75.28068	187 m
	Manati 14	24-MAY-11	15:10:53	S0.48750	W75.27912	185 m
	Manati 15	24-MAY-11	15:34:11	S0.49165	W75.28617	185 m
	Manati 16,17	25-MAY-11	9:49:33	S0.56100	W75.22313	196 m
	Manati 18	25-MAY-11	10:01:45	S0.56226	W75.21876	193 m
	Manati 19	25-MAY-11	10:14:21	S0.55989	W75.21931	191 m
	Manati 20	25-MAY-11	10:36:17	S0.55851	W75.22434	190 m
	Manati 21	25-MAY-11	10:48:20	S0.56004	W75.22691	190 m
	Manati 22	25-MAY-11	11:49:24	S0.56786	W75.22519	194 m
	Manati 23	25-MAY-11	12:26:39	S0.57250	W75.22219	192 m
	Manati 24,25	25-MAY-11	13:01:38	S0.56828	W75.23070	190 m
	Manati 26	25-MAY-11	13:19:01	S0.56707	W75.23651	191 m
	Manati 27	25-MAY-11	13:40:29	S0.56671	W75.23017	189 m
	Manati 28	26-MAY-11	9:15:56	S0.58625	W75.22974	182 m
	Manati 29	26-MAY-11	9:39:16	S0.59081	W75.22912	183 m
	Manati 30	26-MAY-11	12:50:53	S0.58524	W75.24651	188 m
	Manati 31	26-MAY-11	13:28:41	S0.57640	W75.25593	191 m
	Manati 32,33	26-MAY-11	15:15:44	S0.59737	W75.24391	189 m
	Manati 34	27-MAY-11	9:19:56	S0.59670	W75.23789	182 m
	Manati 35	27-MAY-11	9:48:52	S0.60239	W75.23257	183 m
	Manati 36	27-MAY-11	10:20:37	S0.60050	W75.23148	185 m
	Manati 37,38	28-MAY-11	11:44:14	S0.92887	W75.25087	163 m
	Manati 39	28-MAY-11	11:49:20	S0.92793	W75.25268	163 m
	Manati 40	28-MAY-11	15:53:30	S0.92600	W75.25485	188 m

Table 4: This table shows the IDs of the preliminary manatee observations, date and time, latitude and longitude, and altitude. It includes sonar and visual observations.

A total of 83 recordings and 43 screen captures were taken during the three expeditions. Of the 83 recordings, 63 contained usable sonar survey data. Some recordings had no data or it was corrupt. All 63 usable recordings and 43 screen captures (possible manatee detections minus visual sightings) were reviewed as described in the Data Analysis section to assess preliminary manatee detections and determine if false negatives or positives were encountered. Of the 63 sonar recordings with reviewable data, 10 were missing time and date stamps. See Appendix III for the list of recordings and snapshots with time, date, length of recording, and date reviewed. After the sonar recordings and screen captures were reviewed, there were 22 sonar observations and 2 visual observations. None of the 22 sonar detections were visually confirmed by observers in the field. Thirty-nine field observations were not confirmed to be manatees because they did not fit the side-scan model based on body position (signature peanut shape and shadow produced, See Figure 13), eighteen manatee observations which fit the model were observed in the lab review, and two manatees were visually observed while not using sonar. See Appendix II for Humminbird® recording maps. Table 5 presents a summary of manatee sightings after lab review of Humminbird® recordings and screenshots. Table 6 shows that manatees were more frequently observed in Cuyabeno Wildlife Reserve than in Yasuni or Lagartococha.

Table 5: Total Field and Lab Manatee Observations

	Manatee Number	Approx Length (m)	Distance from Boat (m)	Lateral Range (m)	Number of Manatees	Latitude	Longitude	Date/Time (EST)
Yasuni	1	1.5	3.0	6.0	1	0º 57.940S	75º 25.963W	3/6/11 10:42
	2	2.1	7.1	10.1	1	0º 59.578S	75º 26.054W	3/7/11 09:10
	3 4	1.9 2.2	0.1 1.7	14.9	2	0º 59.693S 0º 59.703S	75º 26.932W 75º 26.934W	3/7/11 10:04 3/7/11 10:05
	5	2.1	2.0	4.5	1	0º 31.715S	76º 26.481W	3/10/11 13:06
Lagartococha	6	1.4	1.8	14	1	0º 28.559S	75º 20.745W	5/23/11 13:57
	7	1.7	12.6	14.9	1	0º 30.163S	75º 18.413W	5/24/11 14:18
	8	1.4	1.5	15	1	0º 34.361S	75º 13.280W	5/25/11 13:24
	9	2.8	12.4	15	1	0º 35.867S	75º 13.817W	5/27/11 11:07
	10 11	- -	1.5 2.7	16.7	1	0º 29.318S	75º 16.834W	5/24/11 15:03
	12	1.7	5.6	15.2	2	0º 29.250S	75º 16.760W	5/24/11 15:10
	13	1.4	10.1	16.7	1	0º 29.643S	75º 17.224W	5/24/11 15:44
Cuyabeno	14	1.7	2.1	22.8	1	0º 01.488N	76º 12.220W	7/10/11 13:07
	15 16 17	2.1 2.3 1.6	8.4 5.4 6.5	15.2	3	0º 00.278N 0º 00.277N 0º 00.278N	76º 12.166W 76º 12.152W 76º 12.149W	7/11/11 10:53
	18	1.6	4.3	15.2	1	0º 00.016N	76º 12.418W	7/11/11 11:24
	19	2.1	5.5	15.2	1	0º 00.152N	76º 12.193W	7/11/11 11:12
	20	1.7	10.7	38.4	1	0º 00.906S	76º 13.057W	7/12/11 11:06
	21	1.5	4.6	6.2	1	0º 00.242S	76º 12.558W	7/12/11 11:57
	22	2.5	9.0	30.5	1	0º 00.243S	76º 10.788W	Date and Time Corrupt
	23 24	- -	N/A	N/A	2	0º 33.3018S	75º 13.702W	5/23/11 09:28

Table 5: This table shows the IDs of the field and lab manatee observations, distance from boat, lateral range, number of manatees, latitude and longitude, and date and time recorded. This list includes 4 field sonar observations, 18 lab sonar observations, and 2 visual observations.

Table 6: Probability of Detection of Manatees by Region

Survey Area	Hours Surveyed (hh:mm:ss)	Manatee Detections (visual + sonar)	Probability of Detection (manatees/h)
Yasuni National Park	24:34:30	5	0.203
Lagartococha	28:35:47	10	0.350
Cuyabeno Wildlife Reserve	17:38:46	9	0.510
Total	70:49:03	24	0.338

Table 6: This table shows the number of manatees observed per hour of survey time for each study area.

IMAGES OF MANATEE DETECTIONS

Figure 14 shows Manatee 1 which was not identified in the field survey, but was observed during the Humviewer® review. This manatee exhibits a dark grey color on the side closest to the boat and a decreased acoustic response indicated by the lighter grey on the side away from the boat. There is a weak peanut outline visible in the echo sounder response, the light grey shadow produced in the benthic side-scan return exhibits the more characteristic manatee shape, and the approximate length is 1.5 m. This manatee follows the model of a manatee close to the zero line situated somewhere between the boat and the river bed (See Figure 13A/B). It was positioned at an angle nonparallel to the boat.

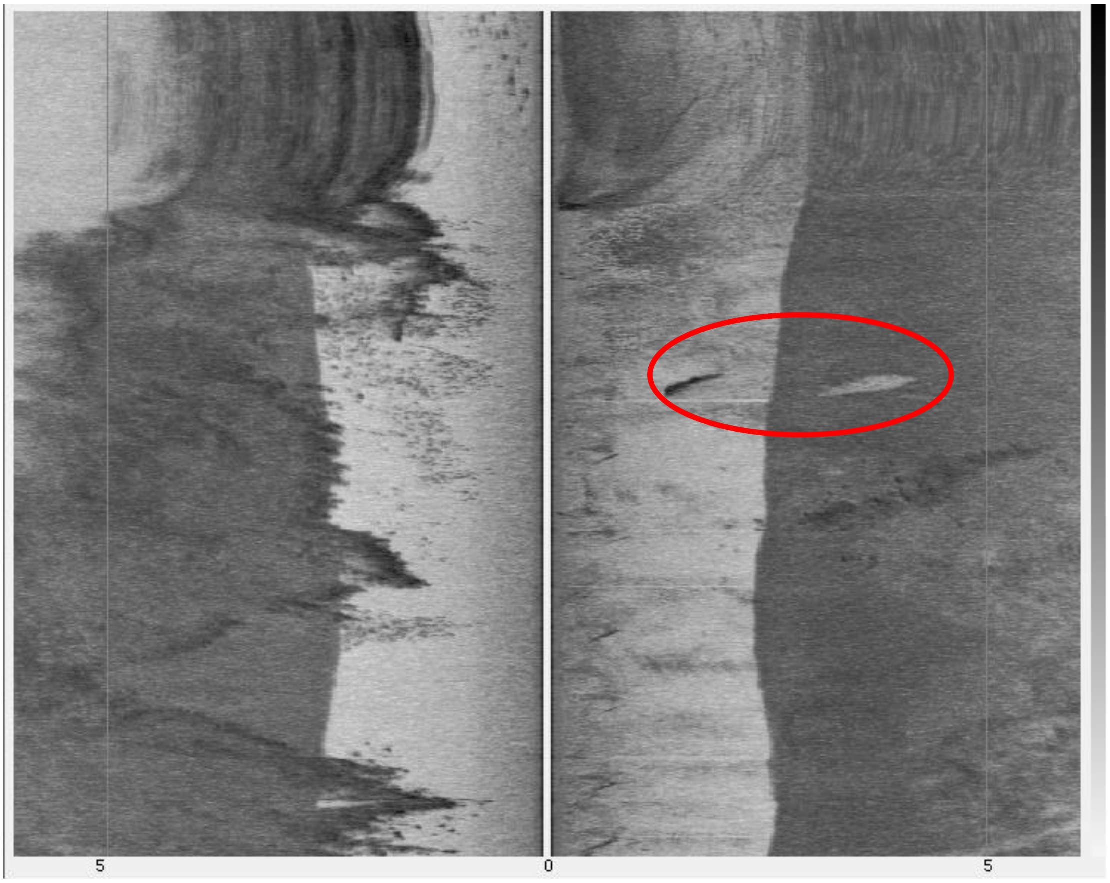


Figure 14: Manatee 1

This is a side-scan sonar image showing a detected manatee (dark grey) and its shadow (light grey) circled in red. The numbers at the bottom show lateral range in meters from the center line (boat position and trajectory). The color scale on the right indicates the appearance of weak (bottom) and strong (top) acoustic responses.

Figure 15 depicts Manatee 2 not identified in the field, but observed during the lab review using Humviewer®. The color settings were changed to blue scale during this review. This manatee exhibits a strong acoustic response on the side of the body closest to the boat indicated by the light blue color. The strong response exhibits manatee morphology with head, flippers, and paddle shape of the tail visible in the outline and the approximate length is 2.1 m. In addition, the characteristic shadow produced by a manatee is present. This manatee observation is similar to the angled body position side-scan model in Figure 13F.

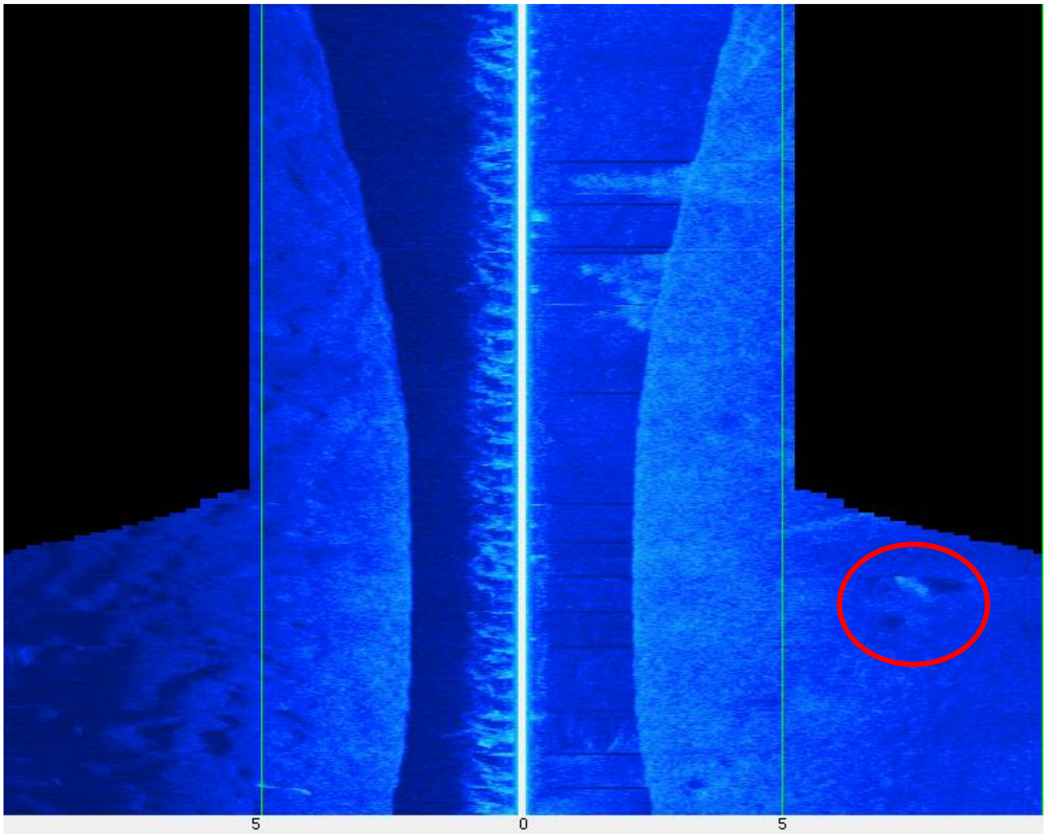


Figure 15: Manatee 2
This is a side-scan sonar image showing a detected manatee (light blue) and its shadow (dark blue) circled in red. The numbers at the bottom show lateral range in meters from the center line (boat position and trajectory). The black areas visible in this image show a decrease in lateral range by the observer.

Figure 16 shows Manatees 3 and 4 missed by the sonar observer, but observed during the lab review using Humviewer®. Manatee 3 has a strong light blue acoustic response present on the side of the manatee body closest to the boat and a dark blue shadow associated with the side away from the boat. The manatee morphology peanut shape is present and the approximate length is 1.9 m. This detection is similar to the model in Figure 13E. Manatee 4 has a strong light blue acoustic response present on the side of the body closest to the boat. This manatee is situated perpendicular to the boat with the head closest to the zero line and exhibits peanut shaped morphology in the outline of the bright response. There is a narrow dark blue shadow produced on the side of the body away from the boat and the approximate length of the manatee is 2.2 m. This manatee detection is similar to the model in Figure 13D, however, was situated much closer to the boat than the model.

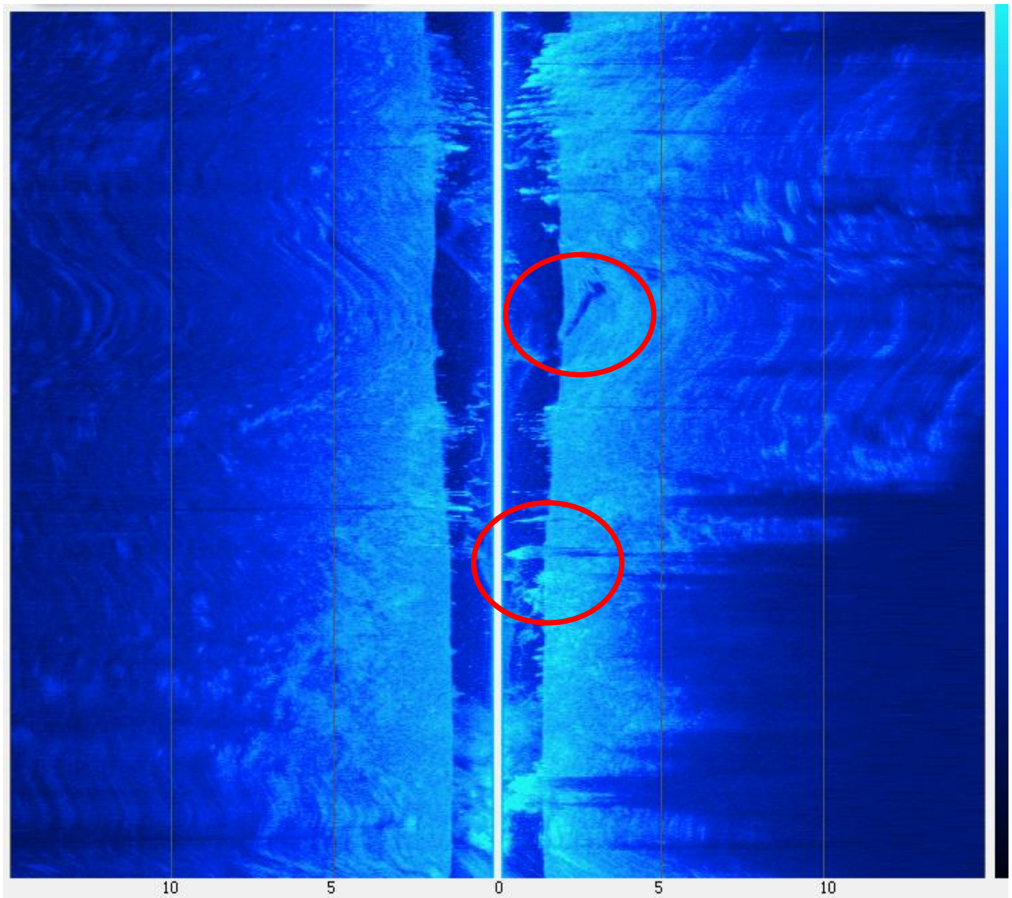


Figure 16: Manatees 3 and 4
This is a side-scan sonar image showing two detected manatees: manatee 3 situated almost parallel to the boat, close to the top of the image (light blue) and its shadow (dark blue) and manatee 4 situated perpendicular to the boat, close to the center of the image (light blue) and its shadow (narrow dark blue) both circled in red. The numbers at the bottom show lateral range in meters from the center line (boat position and trajectory). The color scale on the right indicates the appearance of weak (bottom) and strong (top) acoustic responses.

Figure 17 depicts Manatee 5 not identified in the field, but observed during the lab review of using Humviewer®. This detection demonstrates a strong light blue response on the side of the manatee's body closest to the boat and a dark blue shadow on the opposing side. The outline of the light acoustic response exhibits manatee peanut shaped morphology and the approximate length is 2.1 m. This manatee produced a side-scan image similar to the model of nonparallel, angled manatee position in Figure 13F.

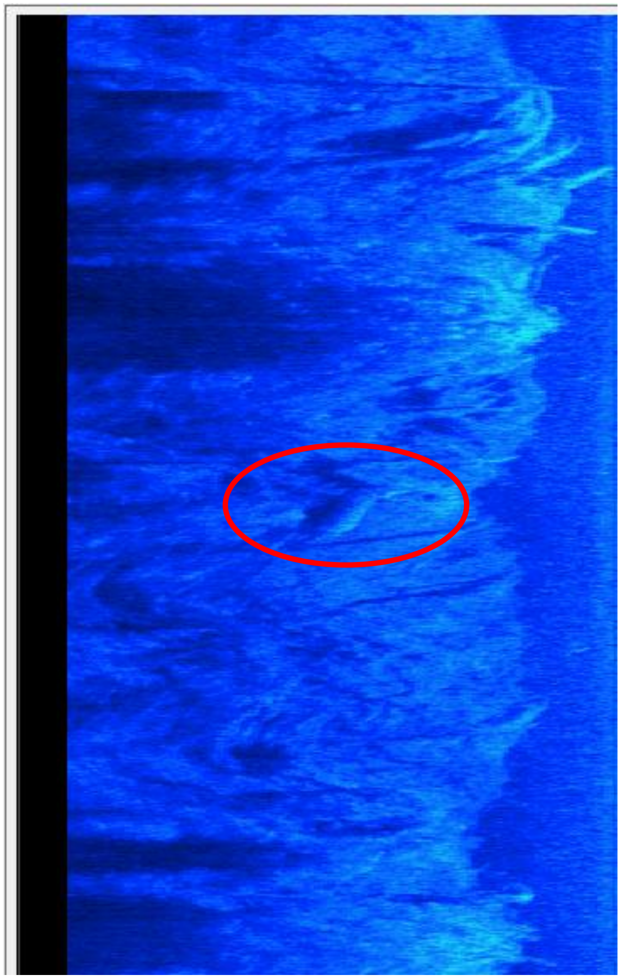


Figure 17: Manatee 5
This is a side-scan sonar image showing a detected manatee (light blue, right) and its shadow (dark blue, left) circled in red. This recording was taken with only the left side of the sonar image and the lateral range scale is not shown.

Manatee 6 (Figure 18) was not identified during the field survey, but was observed during the lab review using Humviewer®. This manatee exhibits a strong acoustic response in light blue on the side of the body closest to the boat. There is a dark shadow produced in the benthic response. This manatee detection is similar to the model in Figure 13A/D. It was situated perpendicular to the boat, therefore, only a portion of the manatee's body produced a response, and its morphology cannot be identified (Figure 13D). The manatee is close to the boat and top of the water column therefore its shadow is shown in the benthic response (Figure 13A). Based on the shadow produced, the approximate length of the manatee is 1.4 m.

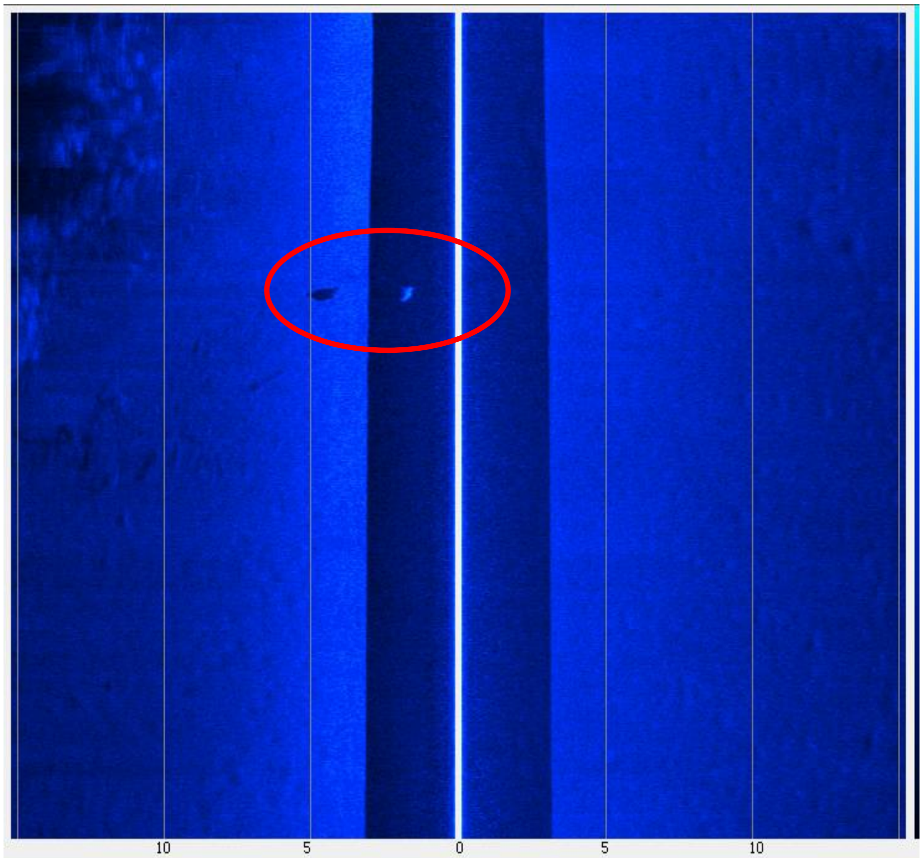


Figure 18: Manatee 6
This is a side-scan sonar image showing a detected manatee (light blue, left) and its shadow (dark blue, far left) circled in red. The numbers at the bottom show lateral range in meters from the center line (boat position and trajectory). The color scale on the right indicates the appearance of weak (bottom) and strong (top) acoustic responses.

Manatee 7 (Figure 19) was not identified during the field survey, but was observed during the lab review using Humviewer®. This manatee is producing a weak, but identifiable acoustic response in light blue on the side of the body closest to the boat. In addition, there is a dark shadow produced on the opposing side of the manatee's body. Signature manatee morphology is visible in the outline of the acoustic response with the paddle and peanut shape and the approximate length is 1.7 m. This detection is similar to the model in Figure 13F with the body positioned at an angle. This manatee was over 10 meters from the boat and the signal and shadow are weak.

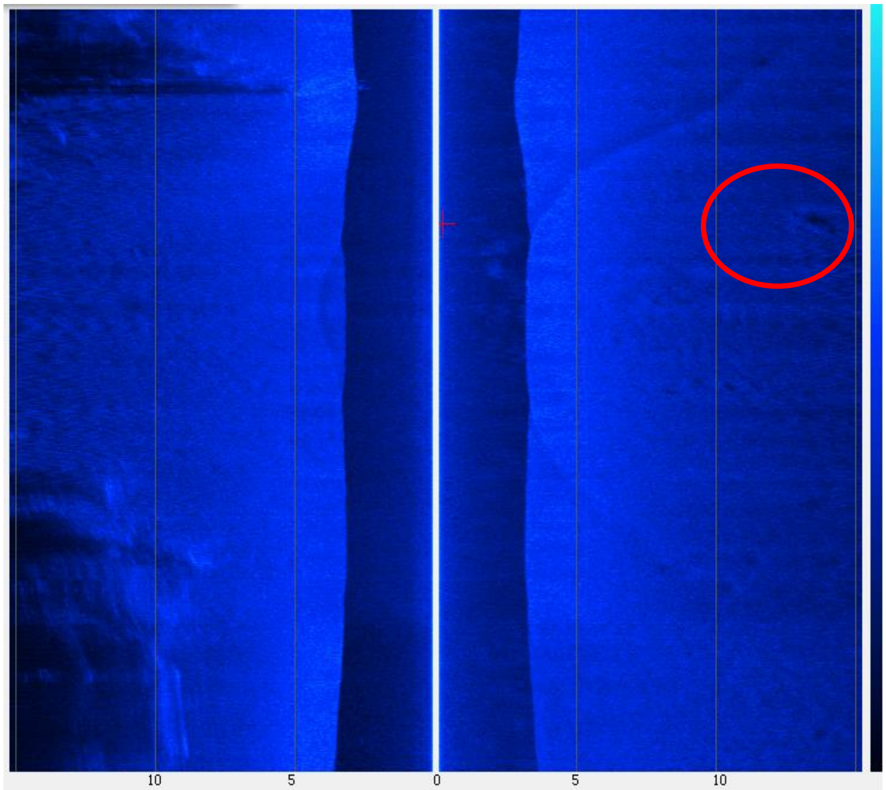


Figure 19: Manatee 7
This is a side-scan sonar image showing a detected manatee (right) and its shadow (far right) circled in red. The numbers at the bottom show lateral range in meters from the center line (boat position and trajectory). The color scale on the right indicates the appearance of weak (bottom) and strong (top) acoustic responses.

Manatee 8 (Figure 20) was not identified during the field survey, but was observed during the lab review using Humviewer®. This manatee exhibits a strong acoustic light blue response on the side of the body closest to the boat in the echo sounder return. There is a strong dark blue shadow produced on the opposing side of the manatee’s body in the benthic return. The morphology of the manatee is clearly visible in the light blue acoustic response with head, paddle, and peanut shape and the approximate length is 1.4 m. This detection is similar to the model in Figure 13A indicating that the manatee was close to the surface of the water and the boat. The shadow is produced far from the manatee in the benthic side-scan response because it is positioned near the topof the water column.

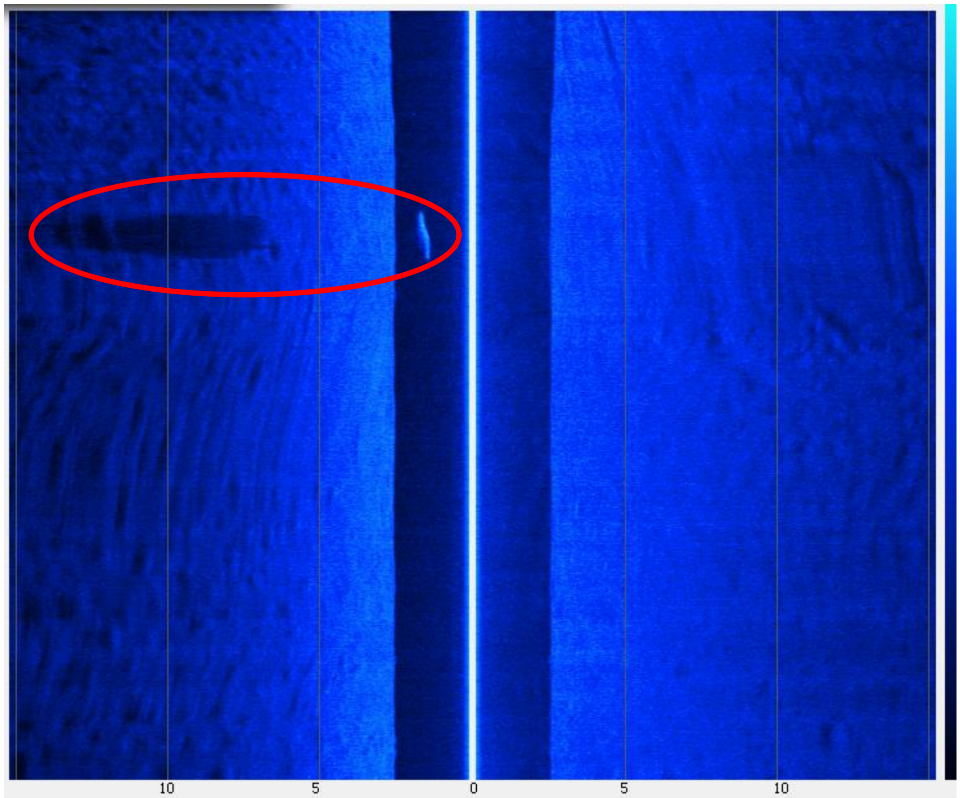


Figure 20: Manatee 8
This is a side-scan sonar image showing a detected manatee (left) and its shadow (far left) circled in red. The numbers at the bottom show lateral range in meters from the center line (boat position and trajectory). The color scale on the right indicates the appearance of weak (bottom) and strong (top) acoustic responses.

Manatee 9 (Figures 21 and 22) was not detected during the field surveys, but was observed during the lab review using Humviewer®. This manatee exhibits a weak, but identifiable acoustic light blue response on the side of the body closest to the boat. The dark blue shadow is present on the opposing side of the body. The manatee peanut-shaped morphology is present and visible in the light blue outline of the acoustic response and the approximate length is 2.8 m. This manatee detection matches the model in Figure 13F at an angle from the zero line, not parallel or perpendicular. There is a weak acoustic response and small shadow because the manatee was over 10 meters from the boat.

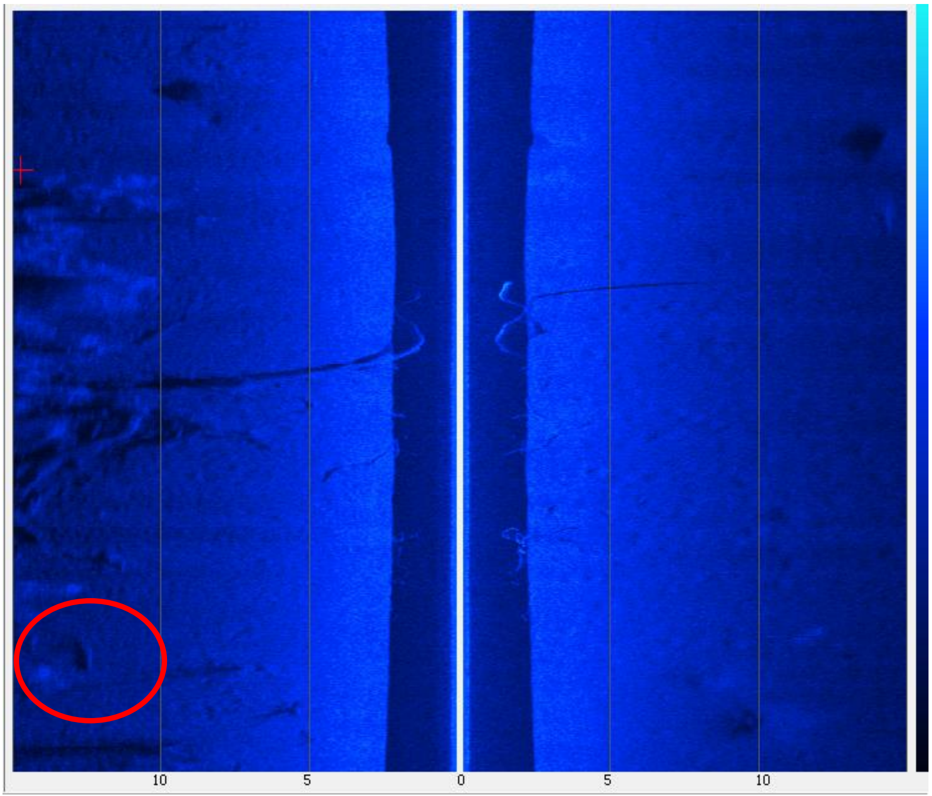


Figure 21: Manatee 9
This is a side-scan sonar image showing a detected manatee (light blue, left) and its shadow (dark blue, left) circled in red. The numbers at the bottom show lateral range in meters from the center line (boat position and trajectory). The color scale on the right indicates the appearance of weak (bottom) and strong (top) acoustic responses.

Figure 22: Manatee 9 Close Up

Figure 23 shows Manatees 10 and 11 which were detected during the field surveys and observed during the lab review using Humviewer®. Manatee 10 closer to the boat exhibits a strong dark grey acoustic response with clearly identifiable manatee morphology. The head, paddle, and peanut shape are visible. There is a bright white acoustic shadow present on the opposing side of the body furthest from the boat. There is a small gap between the manatee's acoustic response and the shadow produced indicating the manatee was closer to the top of the water column. Manatee 11 further from the boat exhibits a strong dark grey acoustic response on the side of the body closest to the boat. The manatee morphology is evident in this detection showing head, peanut shape, and flippers. There is a strong white acoustic shadow produced on the side of the body opposite the boat but is closer to the manatee's body because it was near the bottom of the river bed upon detection. Both detections are similar to the model in Figure 13F with the manatee positioned at an angle from the zero line. The relative sizes of the acoustic responses produced by the manatees could indicate a calf-cow pair. Both manatees produced a disproportionately large acoustic response in this image and approximate length was distorted.

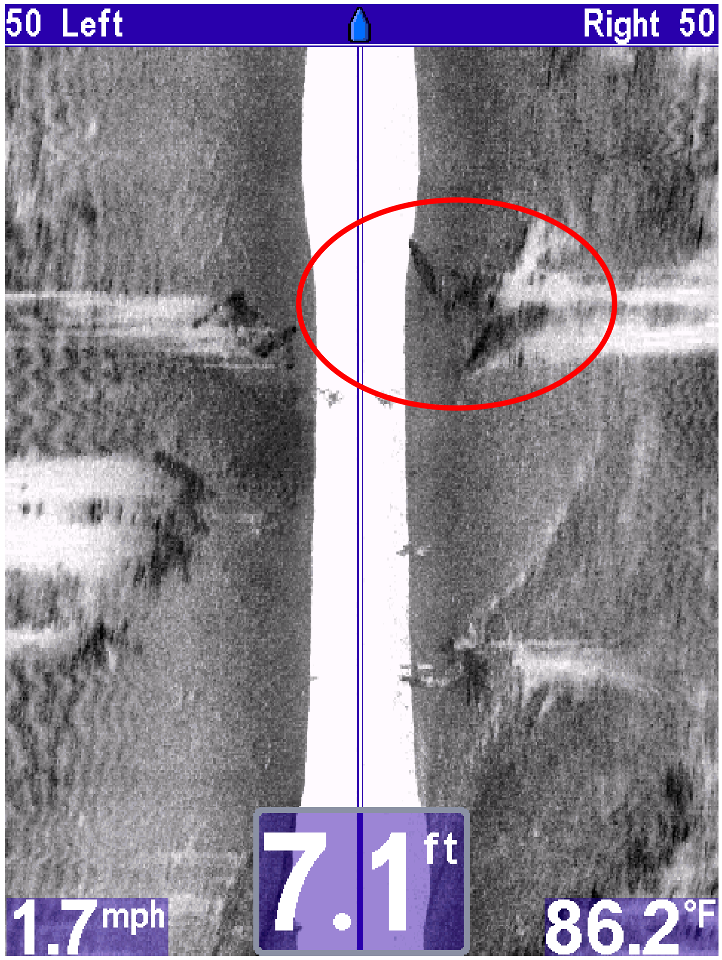


Figure 23: Manatees 10 and 11

This is a side-scan sonar image showing two detected manatees: manatee 10, closer to the center (left), and manatee 11, closer to the right edge and larger (right), both circled in red with bright white shadows. The numbers at the bottom show boat speed, water depth, and water temperature. This is a snapshot taken during a survey.

Manatee 12 (Figure 24) was detected during the field surveys and was observed during the lab review using Humviewer®. This manatee exhibits a strong dark grey acoustic response on the side of the body closest to the boat. The outline of the dark acoustic response demonstrates manatee morphology with the paddle and signature peanut shape and the approximate length is 1.7 m. There is a bright white acoustic shadow visible on the side of the manatee's body opposite the boat. This manatee detection is similar to the model in Figure 13F with the body positioned at an angle from the zero line.

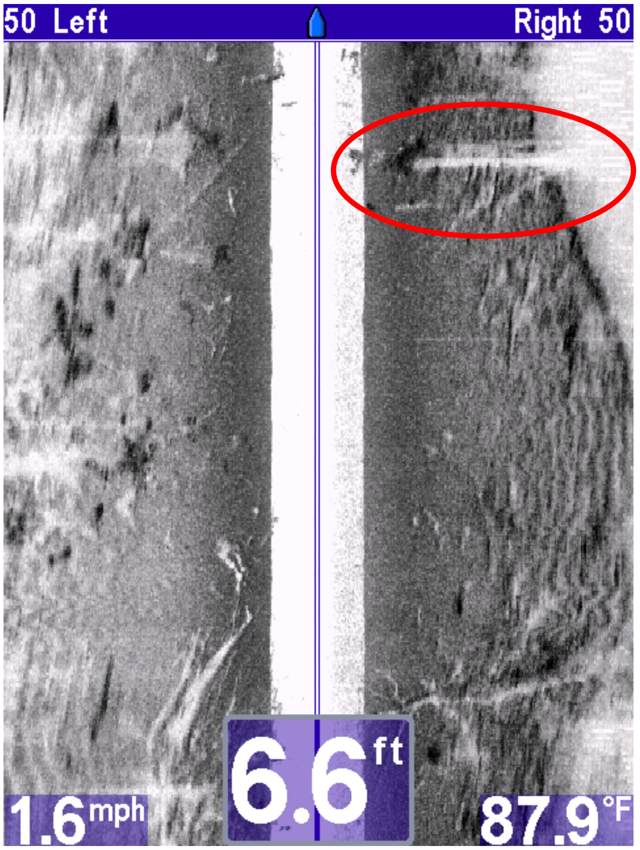


Figure 24: Manatee 12
This is a side-scan sonar image showing a detected manatee (right) and its shadow (narrow white, right) circled in red. The numbers at the bottom show boat speed, water depth, and water temperature. This is a snapshot taken during a survey.

Manatee 13 (Figure 25) was detected during the field surveys and observed during the lab review using Humviewer®. This manatee exhibits a strong dark grey acoustic response on the side of the body closest to the boat. The outline of the dark grey response shows the signature peanut shaped morphology of the manatee and the approximate length is 1.4 m. There is a light grey shadow produced on the side of the body opposing the boat. This manatee detection is similar to the model in Figure 13E with its body positioned parallel to the zero line. The acoustic response is weak and the shadow produced is small because the manatee was greater than 10 meters from the boat.

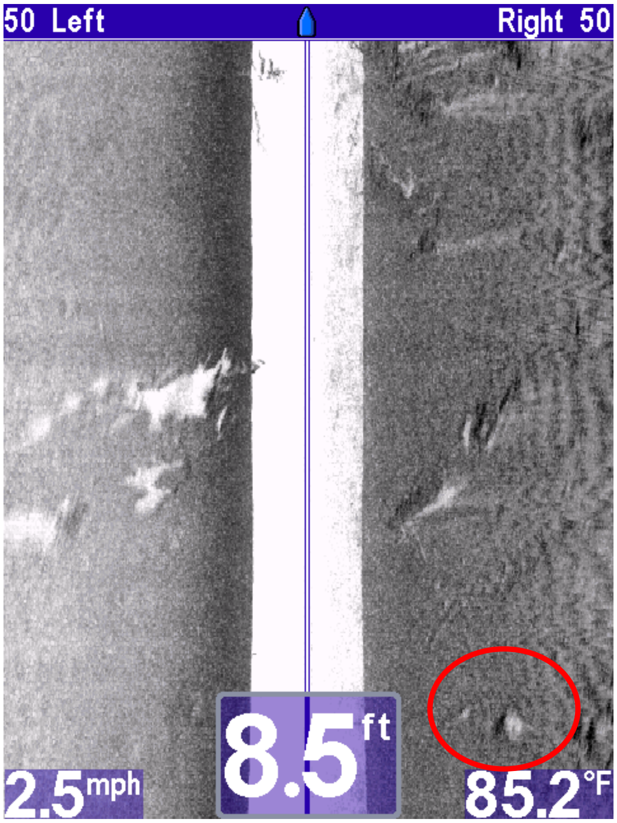


Figure 25: Manatee 13
This is a side-scan sonar image showing a detected manatee (dark grey, right) and its shadow (white, right) circled in red. The numbers at the bottom show boat speed, water depth, and water temperature. This is a snapshot taken during a survey.

Manatee 14 (Figure 26) was not detected during the field surveys but was observed during the lab review using Humviewer®. This manatee exhibits a light blue acoustic response on the side of the body closest to the boat. There is a dark blue shadow produced on the opposite side of the manatee’s body. The signature peanut shape is visible in the outline of the light blue acoustic response and the approximate length is 1.7 m. This manatee detection is similar to the model in Figure 13F with its body situated at an angle from the zero line. This area was heavily vegetated and produced some interference in the image, but this manatee matches the model. There are some bright blue acoustic responses with shadows evident in the middle portion of the image. These are not manatee detections because the outline is jagged indicating vegetation. The manatee detection has a smooth outline consistent with the body shape of a manatee.

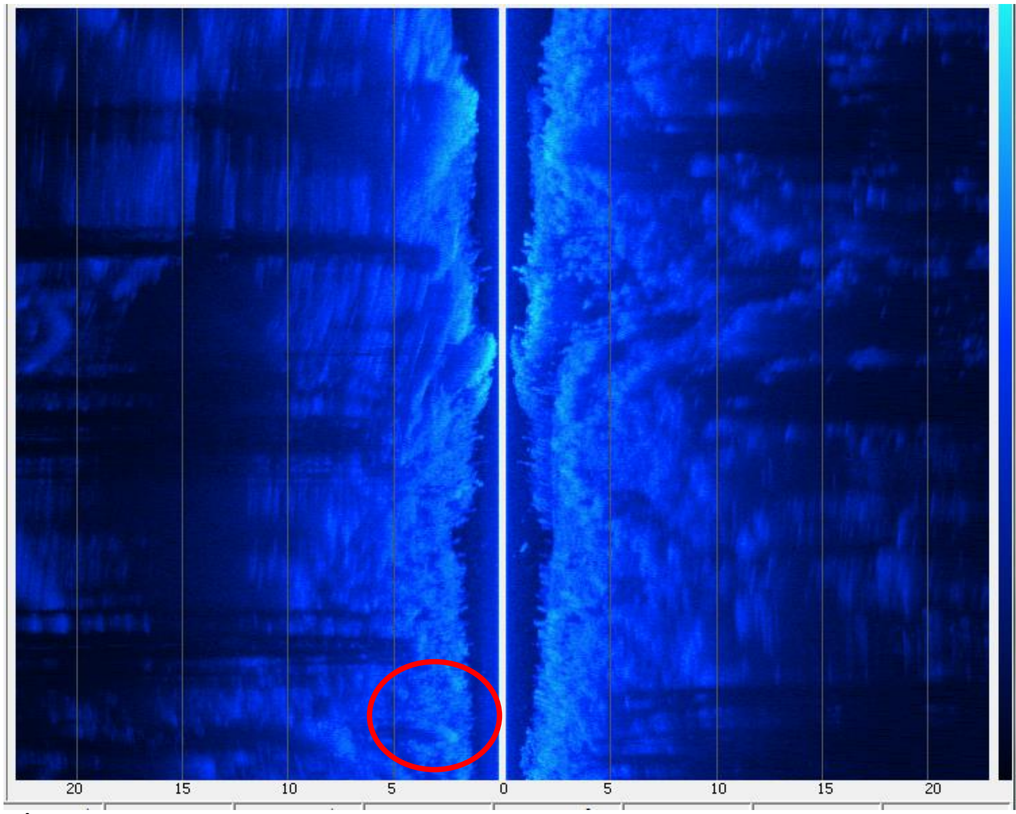


Figure 26: Manatee 14
This is a side-scan sonar image showing a detected manatee (light blue, left) and its shadow (dark blue, left) circled in red. The numbers at the bottom show lateral range in meters from the center line (boat position and trajectory). The color scale on the right indicates the appearance of weak (bottom) and strong (top) acoustic responses. This manatee was observed in an area with a lot of vegetation which caused interference in the image.

Manatees 15, 16, and 17 (Figure 27) were not detected during the field surveys, but were observed during the lab review using Humviewere®. These manatees exhibit similar acoustic responses. They all produce a light blue acoustic response on the side of the manatee's body closest to the boat. All three detections exemplify the manatee morphology including the signature peanut shape, head, and paddle. The approximate length of each observation is 2.1 m, 2.3 m, and 1.6 m. There is a dark blue acoustic shadow associated with all detections on the side of each manatee's body furthest from the boat. These manatee detections are consistent with the model in Figure 13F with the manatees positioned at an angle from the zero line.

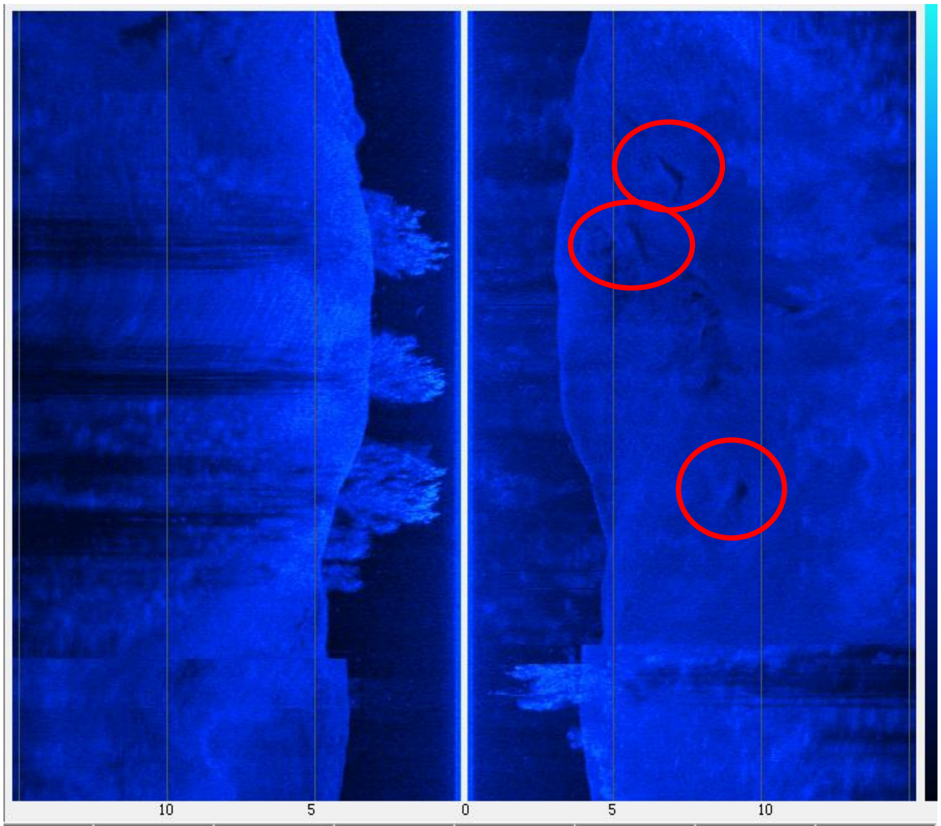


Figure 27: Manatees 15, 16, and 17
This is a side-scan sonar image showing three detected manatees (light blue, right) and their shadows (dark blue, right) circled in red. The numbers at the bottom show lateral range in meters from the center line (boat position and trajectory). The color scale on the right indicates the appearance of weak (bottom) and strong (top) acoustic responses.

Manatee 18 (Figure 28) was not detected during the field surveys, but was observed during the lab review using Humviewer®. This manatee displays a strong light blue acoustic response consistent with the morphology of a manatee and the approximate length is 1.6 m. The head, paddle, and peanut shaped body are visible in the outline of the acoustic response. There is also a long dark blue shadow associated with the side of the manatee's body opposite the boat. This detection is consistent with the model in Figure 13F with the manatee positioned at an angle from the zero line horizontally. There is also similarity to Figure 13C in the model because the shadow is narrower than most. This indicates that the manatee was positioned at angle from the zero line vertically.

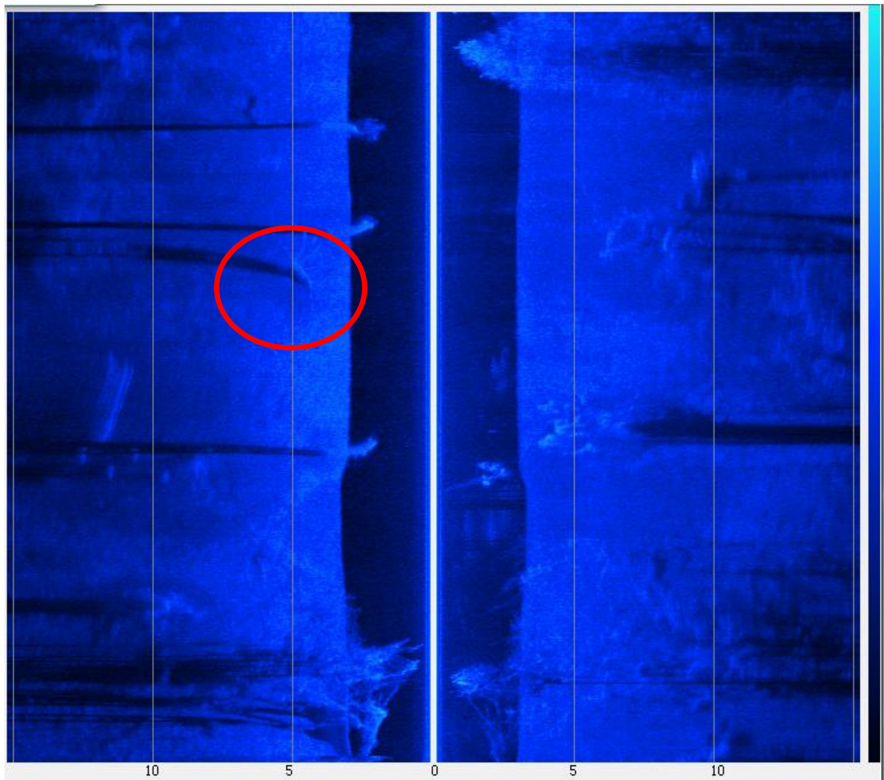


Figure 28: Manatee 18

This is a side-scan sonar image showing a detected manatee (light blue, left) and its shadow (narrow dark blue, left) circled in red. The numbers at the bottom show lateral range in meters from the center line (boat position and trajectory). The color scale on the right indicates the appearance of weak (bottom) and strong (top) acoustic responses.

Figure 29 shows Manatee 19, not detected during the field surveys, but observed during the lab review using Humviewer®. This manatee detection exhibits a strong light grey acoustic response on the side of the manatee's body closest to the boat. This image was taken with the "normal" contrast setting with light acoustic response/dark shadow. The signature shadow in dark grey was also apparent on the side of the manatee's body opposite the boat. The light grey acoustic response demonstrated the manatee morphology with the peanut shape and head and the approximate length is 2.1 m. This manatee detection is similar to the model in Figure 13F with the manatee's body orientation at a slight angle from parallel to the zero line. The greater depth of the water in this region caused a disproportionate amount of the sonar image to be taken up by the echo sounder response. Due to this, the benthic return from the side-scan frequency was constructed in less image space. This caused the shadow to appear shorter than other detections.

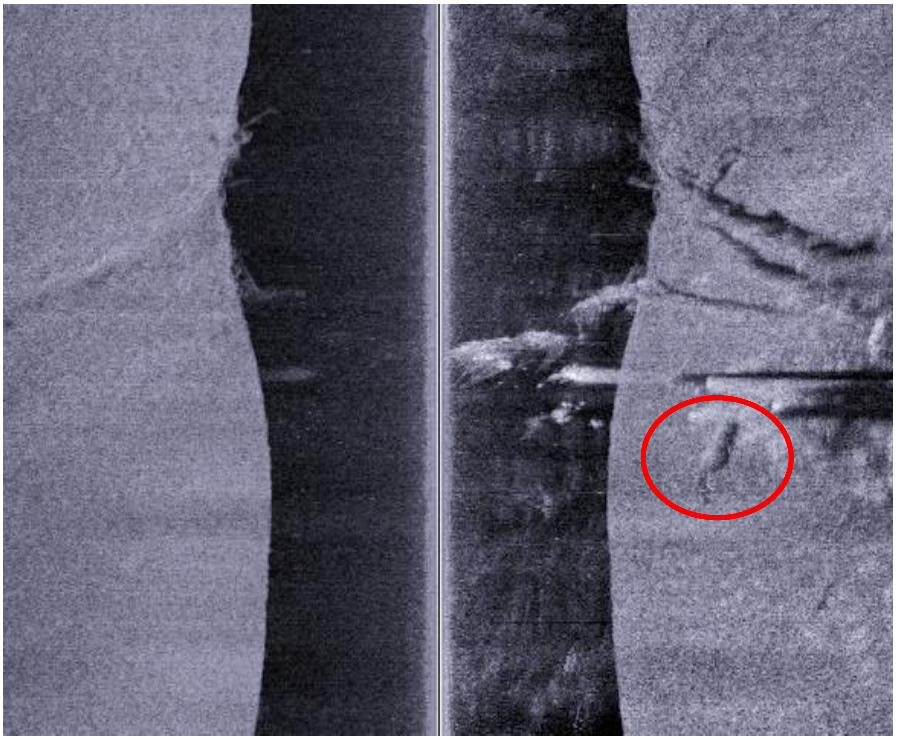


Figure 29: Manatee 19
This is a side-scan sonar image showing a detected manatee (light grey, right) and its shadow (dark grey, right) circled in red.

Manatee 20 (Figure 30) was not detected during the field surveys, but was observed during the lab review using Humviewer®. This manatee detection exhibits a weak, but identifiable light blue acoustic response on the side of the body closest to the boat. There is a dark blue shadow on the opposite side of the manatee's body. The acoustic response demonstrates the manatee morphology with the head, paddle, and signature peanut shape and the approximate length is 1.7 m. This detection is similar to the model in Figure 13E with its body situated almost parallel to the zero line. The lateral range of this survey was 40 meters and the manatee was over 10 meters from the boat. This caused the acoustic response to be weaker than other detections and makes the manatee appear small in the image.

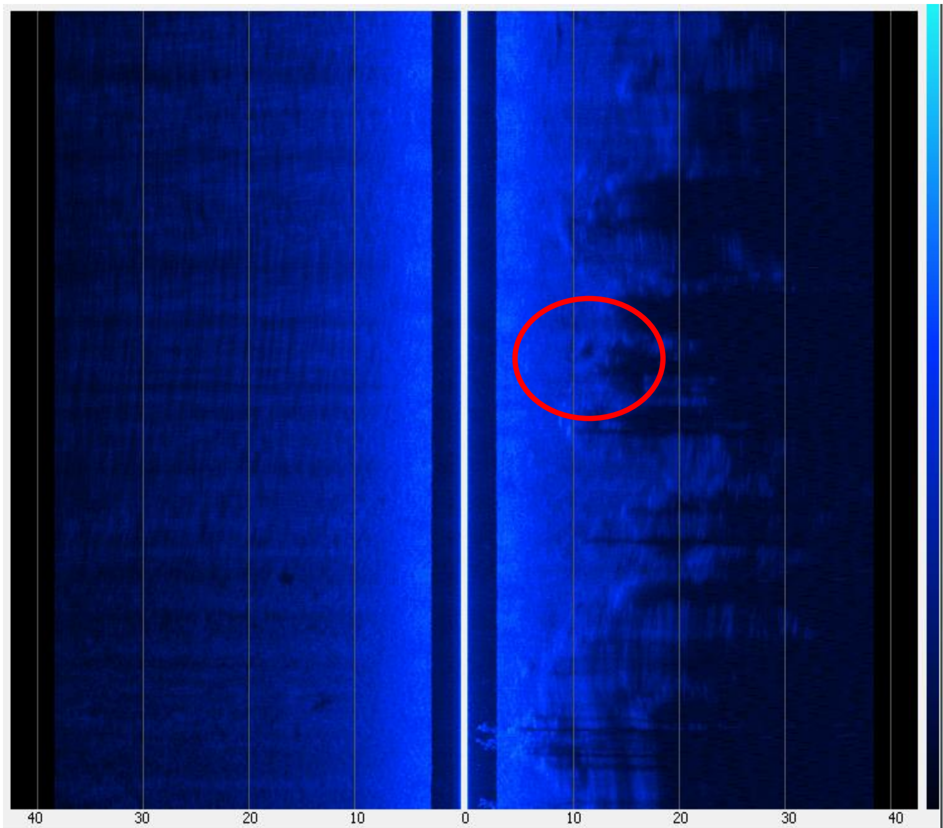


Figure 30: Manatee 20
This is a side-scan sonar image showing a detected manatee (light blue, right) and its shadow (dark blue, right) circled in red. The numbers at the bottom show lateral range in meters from the center line (boat position and trajectory). The color scale on the right indicates the appearance of weak (bottom) and strong (top) acoustic responses.

Figure 31 displays Manatee 21, which was not detected during the field surveys but observed during the lab review using Humviewer®. This manatee exhibits a strong light blue acoustic response on the side of the body closest to the boat. The opposite side of the manatee's body demonstrates a dark blue acoustic shadow. This manatee has apparent peanut morphology; however, it is almost perpendicular to the zero line. Therefore, the acoustic response is capturing only a portion of the manatee's body due to orientation. The approximate length of the observation is 1.5 m. This manatee detection is similar to the model in Figure 13D demonstrating the acoustic response produced by a manatee oriented perpendicular to the zero line.

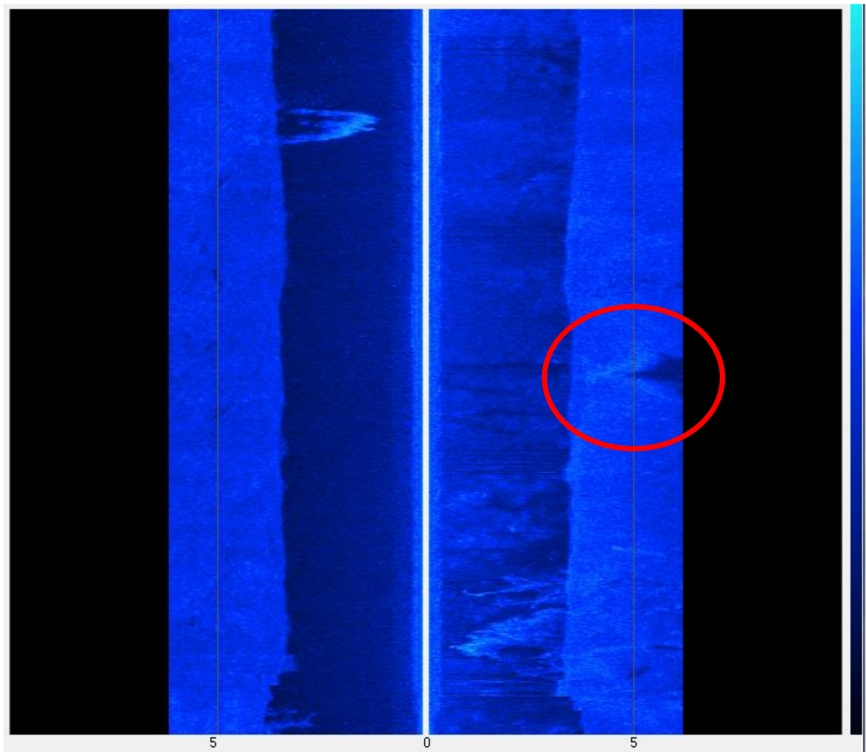


Figure 31: Manatee 21
This is a side-scan sonar image showing a detected manatee (light blue, right) and its shadow (dark blue, right to outside lateral range) circled in red. The numbers at the bottom show lateral range in meters from the center line (boat position and trajectory). The color scale on the right indicates the appearance of weak (bottom) and strong (top) acoustic responses.

Manatee 22 (Figure 32) was not detected during the field surveys, but was observed during the lab review using Humviewer®. This manatee demonstrates a strong light blue acoustic response on the side of the manatee closest to the boat. The signature peanut shaped morphology is present with paddle and head and the approximate length is 2.5 m. There is a dark blue acoustic shadow on the opposing side of the manatee’s body. This manatee is similar to the model in Figure 13F because it is oriented at an angle from parallel to the zero line. This portion of the sonar recording had a lateral range of 25 meters, and because the manatee 10 meters from the boat the acoustic response is weak and the relative size is smaller than other detections.

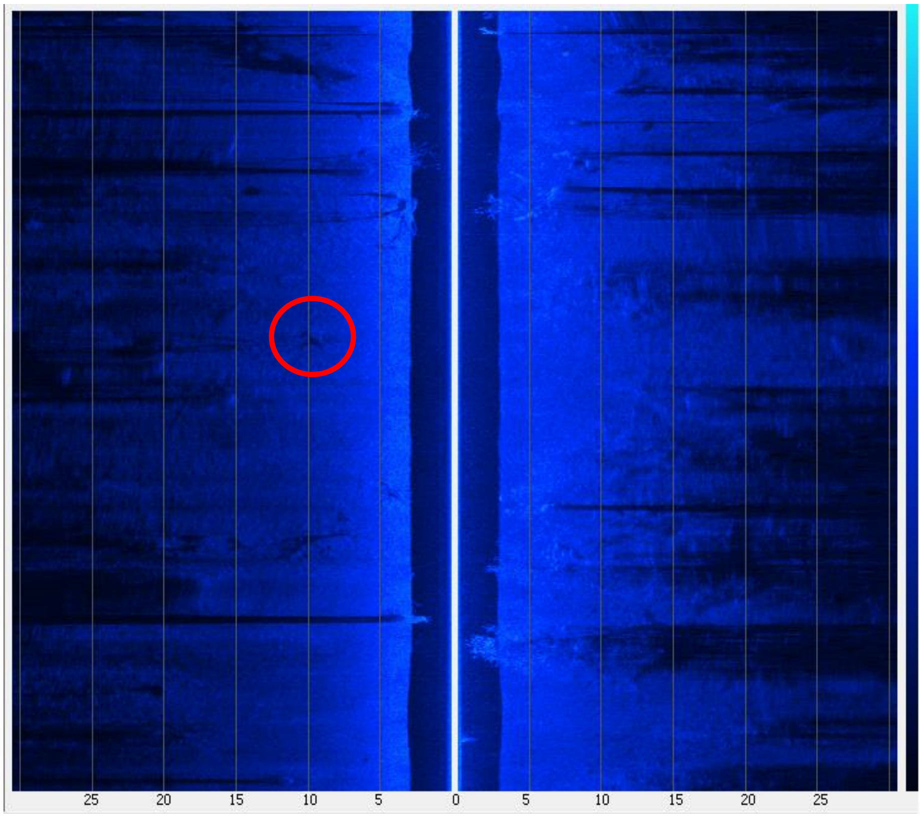


Figure 32: Manatee 22

This is a side-scan sonar image showing a detected manatee (light blue, left) and its shadow (dark blue, left) circled in red. The numbers at the bottom show lateral range in meters from the center line (boat position and trajectory). The color scale on the right indicates the appearance of weak (bottom) and strong (top) acoustic responses.

Final manatee observations were stratified into 5 groups by distance from the boat: 0-3 m, 3-6 m, 6-9 m, 9-12 m, and 12-15 m. Figure 33 demonstrates that the number of detections decreased with increasing distance from the zero line. The visual detections were not included because distance was not measured.

Figure 33: Manatee Sonar Observations by Distance

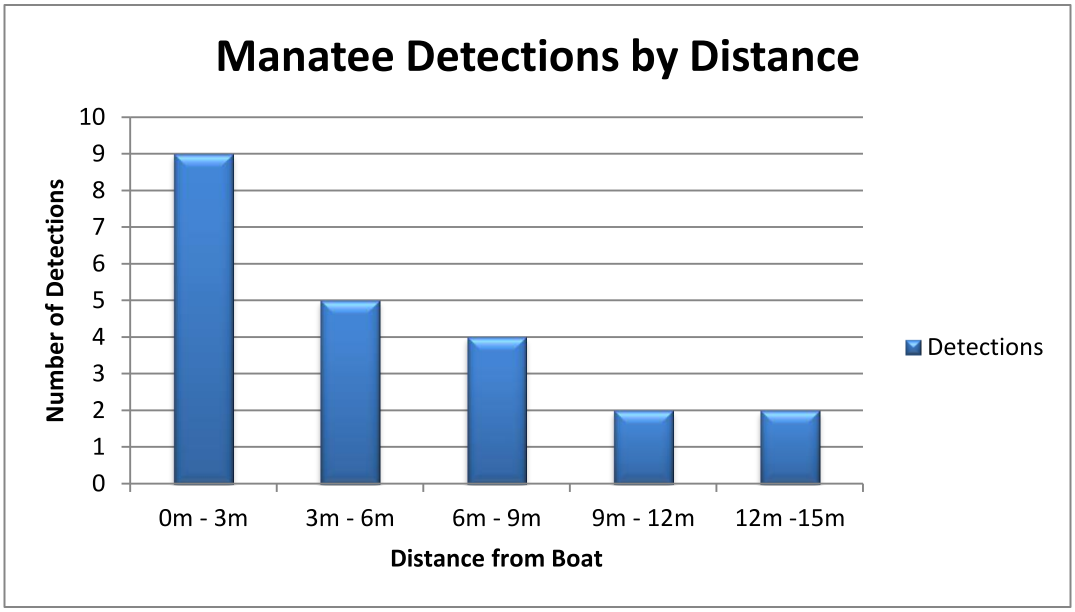
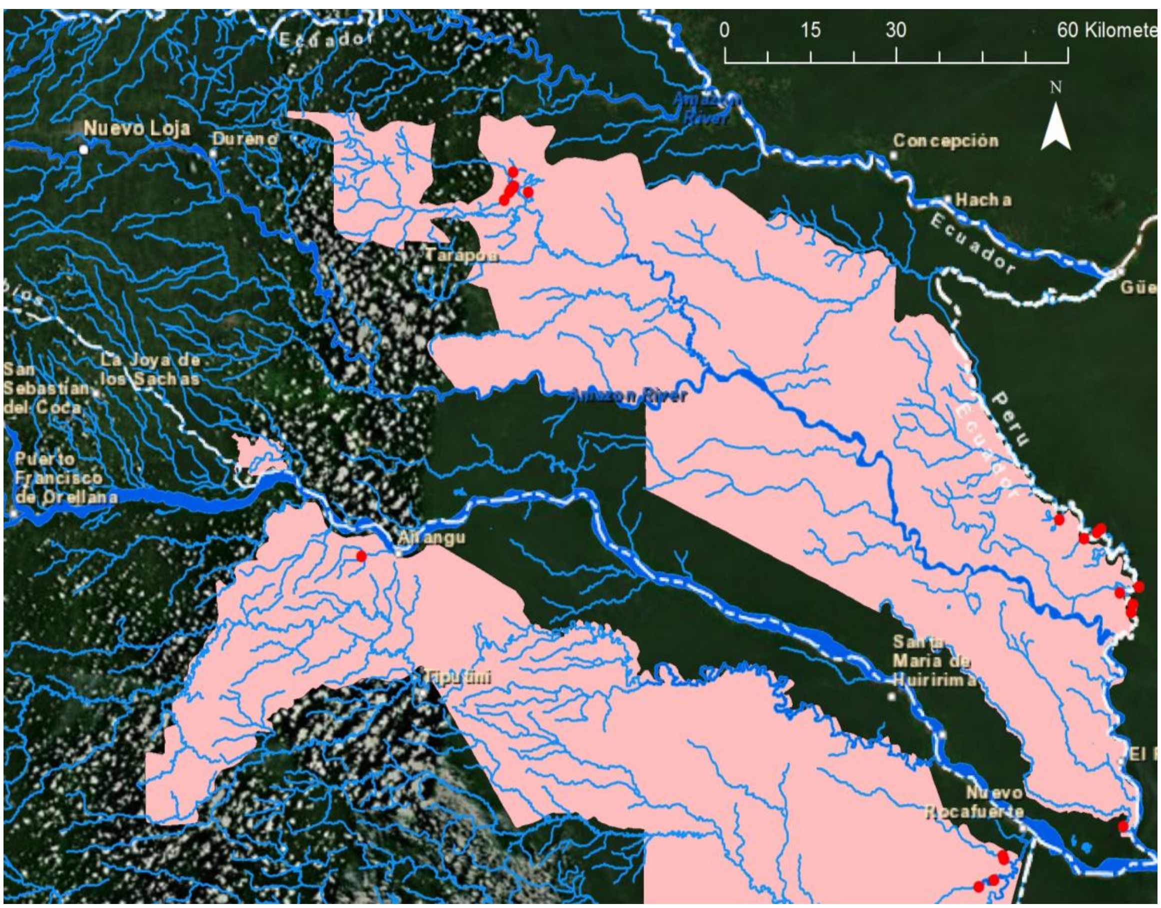


Figure 34: Overview Map of All Manatee Sightings
Red indicates a manatee detection.



DISCUSSION

PRESUMPTIVE AND CONFIRMED MANATEE OBSERVATIONS

Out of the 43 presumptive manatee observations identified in the field, 4 fit the inclusion criteria. Eighteen manatees were observed during the Humviewer® review of survey recordings in the lab. The final number of manatee observations was 24; this number includes 4 field observations, 18 lab observations and 2 visual observations. The data support the hypotheses that the Amazonian manatee persists in Ecuador and side-scan sonar is a viable method for detection. The 39 false field observations and 18 false negatives identified in the lab indicated that there were problems with field detection. This study relied heavily on the lab review with Humviewer® and this left no possibility for confirming observations visually.

There are several reasons which explain the noticeable difference between presumptive and confirmed manatee detections. The Humminbird® unit which displays the sonar acoustic response has a 12.5 cm x 7.5 cm screen. This is quite small and has a limited resolution (as defined by vertical pixels) unless the zoom function is used in the field (Humminbird 797c2 Manual 2006). But using the zoom function in the field is impractical. The zoom can only be used on prerecorded surveys and is limited to a small portion of the recording. This means that the entire image cannot be viewed at a greater resolution while recording surveys. In addition to having a limited size and resolution, the glare from the sun on bright days makes it challenging to see the images on the screen. In Ecuador, the sun can be bright due to latitude and proximity to the sun.

As previously stated in “Image Interpretation” and as described in “Results”, the confirmation of manatee sonar observations requires detailed interpretation and analysis especially in the complex and heavily vegetated Amazon River habitat. For several reasons, this was not always possible in the field. First, the images are only displayed for 10 seconds before a new section of surveyed riverbed is displayed. This isn’t much time for an observer to review the recording. Secondly, the surveys were conducted over long periods of time with two investigators alternating daily. After several hours of performing surveys, investigator fatigue can become a problem and cause perception bias (Gonzalez-

Socoloske and Olivera-Gomez 2012). Finally, both observers were inexperienced in field surveys utilizing the side-scan sonar technology to detect manatees. Prior to the initiation of the investigation, I was briefed on how to use the system and what manatee detections look like, but I had not yet read protocols (Gonzalez-Socolske *et al.* 2009, Gonzalez-Socoloske and Olivera-Gomez 2012). A great deal of background knowledge and experience are required to be able to interpret the images. Specifically, manatee orientation in relation to boat trajectory greatly influences their acoustic reflectivity and shadows produced (Gonzalez-Socolske *et al.* 2009). The knowledge of the models (Figure 13) of the side-scan sonar acoustic response recorded for a given manatee is essential for field detection.

The detection problems in the field were caused by limited display time and resolution, a small screen, glare from the sun, perception bias due to fatigue, and inexperience of both observers. Given these issues with detecting manatees during field side-scan sonar surveys, the ability to review and evaluate the recordings using the Humviewer® software was essential. Humviewer® displays recordings in greater resolution and detail on a much larger screen, and it allows for slow and detailed review. Instead of being limited to 10 seconds for interpretation, the image can be paused for the observer to compare presumptive detections to models and side-scan sonar images of visually confirmed manatees. Color contrast settings can be changed, zoom can be used to evaluate presumptive detections, and observations can be analyzed for validity. Through this detailed review and comparison, the primary observer gained valuable interpretation experience for future surveys. The downside to missing observations in the field and identifying them during the lab review is that there is no chance for visual confirmation.

There are several recommendations for future side-scan sonar surveys resulting from this study. Performing control studies in clear water habitats where manatees can be visually confirmed will establish familiarity with field detection. Preliminary studies in a controlled setting will increase observer experience with acoustic response interpretation. Inclusion and exclusion criteria for manatees observed via side-scan sonar can be established by reference to specific known samples. Manatees were detectable with

method modifications, but counts are likely conservative and eliminating protocol deviations will improve future side-scan sonar surveys. Adding a sheet for the observer to block the sun could prevent field detection problems in future studies.

EFFICIENCY OF DATA COLLECTION

Twenty-four Amazonian manatees were observed in Yasuni National Park, Lagartococha, and Cuyabeno Wildlife Reserve using the side-scan sonar method for collecting population data. This data was collected in a total of 238.8 km surveyed in 70 hours 49 minutes. Denkinger 2010 performed a survey in Lagartococha and Cuyabeno River using boat-based visual survey methods and no side-scan sonar. The effort for Denkinger (2010) is summarized in Table 7. The 2010 study confirmed 4 manatee observations in 201 km surveyed in 454 hours. The data support the hypothesis that the effort required for visual, boat-based surveys without the aid of side-scan sonar is exponentially greater than that required for this survey.

Not only do visual surveys require more effort, the elusiveness of the Amazonian manatee, poor visibility in the black, turbid water of the Amazon, and heavy vegetation produce fewer detections. Timm (1986) and Denkinger (2010) performed visual surveys, had 10 and 4 observations respectively, and relied heavily on second hand accounts. The fact that only 2 manatees were visually observed during this survey further supports the hypothesis. In conclusion, the results of this study support the hypothesis that side-scan sonar is a more efficient method than visual surveys for gathering population data on the Amazonian manatee. Additionally, our data support the hypothesis that the manatee is elusive.

	Distance Surveyed (km)	# Transects	Hours Surveyed	# Manatees Sighted	Probability of Detection (Manatees/h)
Cuyabeno River - Denkinger	64.9	81	311.95	3	0.010
Cuyabeno River - Brice	58.3	8	17.63	9	0.510
Lagartococha River - Denkinger	136.1	65	142.10	1	0.007
Lagartococha River - Brice	110.3	8	28.58	10	0.350

Table 7: This table compares the effort on surveys in manatee habitat and probability of detecting manatees in the Cuyabeno and Lagartococha Rivers from Denkinger 2010 and this study.

POPULATION ABUNDANCE AND MODELLING

The total number of manatees detected and the probability of detection were compared between the three study areas. In Yasuni National Park, 5 manatees were detected; in Lagartococha, 10 manatees were detected; and in Cuyabeno Wildlife Reserve, 9 manatees were detected. The raw manatee counts suggest that a higher abundance of manatees exist in the Lagartococha study area when compared to the Yasuni and Cuyabeno counts. This supports the hypothesis that manatees are more abundant in Lagartococha than Yasuni and Cuyabeno and may be more abundant in the far Eastern part of the Ecuadorian Amazon near the Peruvian border. However, when the probability of detection is compared, there is a different conclusion. In Yasuni National Park the probability of detection was 0.203 manatees/hr, in Lagartococha the probability of detection was 0.350 manatees/hr, and in Cuyabeno Wildlife Reserve the probability of detection was 0.510 manatees/hr (See Table 6). These data support the hypothesis that more manatees were encountered per hour in Cuyabeno Wildlife Reserve. When compared to probability of detection for Denkinger 2010 (See Table 7), manatees were encountered more often in both Lagartococha and Cuyabeno than previously reported.

The number of manatee detections made utilizing the side-scan sonar system was compared to the distance from the boat when detected. The visual detections were not

included because distance was not measured. When the number of sonar detections was graphed versus the distance from the boat, the number of detections clearly decreased with increasing distance from the zero line (See Figure 33). This supports the hypothesis that the detectability of a manatee using the side-scan sonar system decreases with increasing distance from the transducer. These data support the hypothesis that at least one assumption for using Distance® sampling as an analysis tool has been met.

Although detections decreased with distance from the boat, there are some problems with that conclusion. There was not always manatee habitat up to 15 meters from the transducer in some areas such as narrow tributaries. Since our study was designed with opportunistic, non-straight line transects, it is not certain that manatees were distributed randomly with respect to transect lines. If future surveys aim to use Distance® sampling as an analysis tool, transects should be planned ahead of time and designed to meet the assumptions. It is recommended that surveys be focused in areas where the transducer has full range of detectability (up to 20 m for manatees). Since the study region is a complex habitat and side-scan sonar differs from strictly visual surveys, other population analysis tools should be tested.

CONCLUSIONS

The population of Amazonian manatees in eastern Ecuador is extant, in contrast with the Timm *et al.* (1986) prediction. Results demonstrate that side-scan sonar is a viable method for detecting Amazonian manatees in the Ecuadorian Amazon. Manatees were encountered more often in Cuyabeno Wildlife Reserve than in Lagartococha and Yasuni. The probability of manatee detection was greater in Cuyabeno Wildlife Reserve and Lagartococha than reported in 1996-1999 (Denkinger 2010). Side-scan sonar detected more manatees than previously reported with visual survey methods in Denkinger 2010 and Timm *et al.* 1986. Side-scan sonar resulted in greater detection as a function of effort when compared to visual surveys.

The main problem with research on the population status of the Amazonian manatee in Ecuador is its elusive nature. The data from this study demonstrate that manatees were detected in an area where we assume they cannot be detected. This study

had problems with field detection and relied heavily on laboratory analysis. However, all survey techniques have inherent detection problems. All future population surveys of the Amazonian manatee should incorporate this method because it increases the effectiveness of surveys and is affordable. It is recommended that future studies establish familiarity in a controlled setting prior to use in the field. Observer reliability analyses are recommended as part of an ongoing protocol. All side-scan sonar surveys should be recorded and analyzed in the lab. Side-scan sonar data should be tested for possible use in population monitoring using Distance® and other population models. In the long term, the more Amazonian manatee data that are collected with the side-scan method, the better understanding scientists and researchers will have about the population.

CHAPTER III

WATER SAMPLES AND CHEMICAL ANALYSIS

OBJECTIVES AND HYPOTHESES

The objectives of this study were to:

- Determine if crude oil constituents are present at toxic levels in the habitat of the Amazonian manatee in Ecuador utilizing surface water sampling and analysis.
- Test the alternate hypotheses that the levels of chemical contaminants have increased, decreased, or stayed the same since last measured in 1994 and 1998 by quantifying differences.
- Investigate the hypothesis that pollutant concentrations vary spatially as a function of oil activities.

INTRODUCTION AND BACKGROUND

As previously stated, the Amazonian manatee is classified as vulnerable to extinction worldwide and is severely endangered within Ecuador (Marmontel 2008, CITES 2013, Tirira 2011, Timm *et al.* 1986, Denkinger 2010). The decline is primarily caused by ongoing levels of hunting, sometimes involving new and sophisticated techniques, coupled with increasing incidental calf mortality, climate change, and habitat loss and degradation. These threats are solely anthropogenic in origin because the species has no natural predators (Marmontel 2008).

Industrial development contributes to the habitat loss and degradation that threaten the Amazonian manatee in Ecuador. The tributaries and lagoons of the Amazon River are threatened by an economic boom, primarily attributable to the petroleum extraction industry. The Ecuadorian government has zoned 65 % of its Amazon basin – including wildlife protected areas – with oil blocks in an effort to bring economic resources to the country and deal with increasing debt (See Figure 2, Aaen 2006, Finer *et al.* 2008). The direct impacts of the oil extraction include deforestation for access roads, drilling platforms, and pipelines, and contamination from oil spills and wastewater discharges. Indirect effects arise from the easy access to previously remote primary forest

provided by new oil roads and pipeline routes, causing increased logging, hunting, and deforestation and an increase in boat traffic from human settlement (Finer *et al.* 2008). The pollution, deforestation, and increased boat traffic threaten the manatee's survival along with thousands of other species that inhabit the extremely biodiverse Ecuadorian Amazon (Finer *et al.* 2008).

The practices and technology of oil development activities include several contaminating processes (Sebastian and Hurtig 2004). In Ecuador, exploratory wells are drilled and can produce an average of 4,000 cubic meters of drilling waste including formation water and drilling muds containing lubricants and sealants. Wastes are frequently discharged into open, unlined pits called separation ponds from which they either directly contaminate the environment or leach out as the pits degrade or overflow from rainwater. Some companies created better ways to deal with the wastes, but in 2004 there were over 200 open ponds in the Amazon region (Sebastian and Hurtig 2004).

If commercial quantities of oil are found, production begins and oil is extracted as a mixture of formation water and gas which is separated at a central facility. Each facility can generate as much as 16.3 million liters of untreated liquid waste every day. Annually, routine maintenance activities at over 300 producing wells discharge approximately 18.9 million liters of untreated toxic wastes into the environment. Additionally, roughly 1.5 million cubic meters of waste gas from the process are burned daily without temperature or emission control (Sebastian and Hurtig 2004).

Not only do wastewater discharges from the petroleum industry contribute to toxic contamination, oil spills are common in the Ecuadorian Amazon. Sources of spills include leaks from wells and tanks, connecting flow lines between wells and stations (approx. 75,800 liters every 2 weeks), and main and secondary pipelines that connect separation stations to the refineries in the coastal regions. In 1992, the Ecuadorian government reported an estimated total loss of 63.6 million liters of crude oil in approximately 30 major oil spills. In the Napo River, 1.1 millon liters and 1.0 million liters were spilled in 1989 and 1992 respectively (Sebastian and Hurtig 2004). In 1995, there was a major oil spill in the Cuyabeno Lagoon (Marmontel 2008). Overall, since the discovery of vast amounts of crude oil in 1967 by the Texaco-Gulf Consortium (Aaen

2006, CESR 1994) and subsequent petroleum extraction development (Aaen 2006), from 1972 to 1993, more than 114 billion liters of toxic wastes and crude oil were discharged into the land and waterways of the Ecuadorian Amazon (Oriente) (Sebastian and Hurtig 2004). There has been a spill as recently as January 2008 in Ecuador's Yasuni region (Finer *et al.* 2008) and 1.6 million liters were spilled on June 1st, 2013 into Rio Napo (Solano 2013).

The waste generated and crude oil spilled into the environment contain many chemical contaminants of concern. Drilling wastes, a mixture of oil, natural gas, and formation water deep below the earth's crust contain hydrocarbons, heavy metals, and high concentrations of salt (Sebastian and Hurtig 2004, Sebastian *et al.* 2001). Crude oil is a complex mixture of many chemical compounds, mostly hydrocarbons. Petroleum hydrocarbons of most toxicological interest are VOCs and PAHs (Sebastian *et al.* 2001, CESR 1994).

Heavy metals such as arsenic, lead, or mercury are toxic to mammalian species. They can affect the neurological development of neonates in certain species (rats, monkeys, and humans), but to date, no link has been established in any marine mammal (Belanger and Wittnich 2008). Much of the Sirenian research in the past 30 years has focused on measuring the levels of heavy metals in tissues with little focus on identifying lethal levels or the effects on organs and fetal development (Belanger and Wittnich 2008). Arsenic is toxic to humans; prolonged exposure causes skin, lung, or other cancers and chronic exposure causes skin lesions, peripheral neuropathy, and anemia (Gehle *et al.* 2009). Lead has hematologic, neurologic, renal, and reproductive toxicity to humans (Landrigan 1990). Mercury is toxic to the central nervous system, kidneys, lungs, and gastrointestinal tract in humans (Clarkson *et al.* 2003).

TPH refers to all hydrocarbons present in crude oil. Studies on mice have reported skin tumors after exposure to crude oil, however, there is limited evidence showing carcinogenicity of crude oil in experimental animals and humans (Sebastian *et al.* 2001). Drinking water contaminated with oil has been associated with increased incidence of esophageal cancer. Inhalation of high levels of crude oil fumes can lead to adverse effects on respiratory systems and cause life-threatening chemical pneumonitis and other

systemic effects. In addition, exposure to crude oil may also lead to adverse reproductive and developmental effects (CESR 1994). VOCs are a subset of TPHs that easily evaporate at room temperature; examples include benzene, xylene, styrene, and toluene. Benzene is a well-known cause of leukemia, and other hematological neoplasms and disorders. Limited evidence has associated xylene with increased risk for colorectal cancers, toluene with esophageal and rectal cancers, and styrene with rectal cancer (Sebastian *et al.* 2001). PAHs are another subset of TPHs in crude oil characterized by fused aromatic rings. Direct evidence of carcinogenic effects of PAHs in occupationally exposed human subjects has been reported including skin, bladder, scrotum, and lung toxicity (Sebastian *et al.* 2001). In addition, a prototypic group of 17 PAHs has been linked to adverse health effects ranging from skin irritation to cancers and toxic effects on reproduction and cellular development (CESR 1994)

Direct exposure to PAHs, VOCs, TPHs, and heavy metals due to oil contamination poses serious toxicological and sensory deprivation risks to a manatee. As in other marine groups, it is presumed that exposure to petroleum would irritate eyes and sensitive mucus membranes during respiration behaviors. Their pelage is limited to sparse sinus hair, which plays an important role in cutaneous perception. Coating of these structures with oil could result in significant impairment and irritate eyes and lungs (St. Aubin and Lounsbury 1990). Animal sensory systems are critical to their survival because they detect environmental information. Not only could oil exposure impair the visual, tactile, and nasal sensory system function, chemoreception has been suggested to play an important role in manatee perception (Bills *et al.* 2013). If manatees utilize chemoreception to identify ideal habitats, mates, or food sources then oil exposure could interfere with survival (Bauer *et al.* 2010). It is not inconceivable that manatees might consume tar balls or fresh petroleum accidentally along with their normal diet. The need for manatees to occupy somewhat restricted habitats places them in a potentially vulnerable position, particularly during winter. Oil spills or any other environmental perturbation within the confines of preferred river systems and lagoons would likely endanger the local population (St. Aubin and Lounsbury 1990) especially since Amazonian manatees migrate seasonally to deep water lagoons (Arraut *et al.* 2010).

Previous reports indicate contamination by TPHs, VOCs, and PAHs is reaching a critical level in Ecuador (Sebastian and Hurtig 2004). In 1987, the Ecuadorian Government found elevated levels of TPH in 36 samples from rivers and streams near production sites and that a shortage of dissolved oxygen in the majority of samples had significantly harmed the ecosystem (Sebastian and Hurtig 2004). In 1994, the Center for Economic and Social Rights [CESR] documented the exposure and health risk that pollution from the oil industry has caused for humans. They analyzed thirty-three water samples and found toxic PAHs in 22 samples and VOCs in 5 samples. Some of these toxic chemicals were 10 to 1000 times the legal limits set by the EPA in the United States (CESR 1994). In 1998 an independent local laboratory that is frequently used by the oil companies surveyed 46 streams in the Oriente region. The laboratory found contamination by total petroleum hydrocarbons (TPH) in areas of oil activities, while no water contamination was found in areas without such activities (Sebastian and Hurtig 2004). In 1999, the Instituto de Epidemiología y Salud Comunitaria, a local nongovernmental organization concerned with health issues, undertook water analyses for TPH. In some streams, hydrocarbon concentrations exceeded by more than 100 times the limit permitted by European Community regulation (Sebastian and Hurtig 2004). Since 1999, the oil companies have been required by law to regularly monitor the pollution in the environment and send reports to the Ecuadorian Government. This information is not open for public scrutiny, however, one report from 1999 showed concentrations of TPH at 500 times the limit permitted by European Community regulations. It was insisted by the government that measured levels were acceptable (Sebastian and Hurtig 2004). In 2001, levels of TPH measured were 10 to 288 times higher than the limit permitted by the European Community regulations (Sebastian *et al*. 2001).

Ecuador is a small country that relies on the oil industry for half of its total export earnings and for over one third of its annual federal budget (Bass *et al.* 2010). Oil and gas development in the western Amazon has already caused major environmental and social impacts (Finer *et al.* 2008). Thus, an increase in the scope and magnitude of planned hydrocarbon activity in the habitat of the Amazonian manatee is inevitable and documented contamination issues are likely to intensify (Finer *et al.* 2008).

METHODS AND DESIGN

Water samples were collected in conjunction with the side-scan sonar survey. For a detailed description of the study areas see Chapter II. Samples were collected opportunistically at various sampling points during each side-scan sonar survey. Sampling points were chosen during the surveys based on presumptive side-scan sonar manatee observations, high anthropogenic activity, or close proximity to petroleum extraction development. Nine samples were collected in Yasuni National Park in March 2011, seven samples were collected in Lagartococha, Gueppi Wildlife Reserve and Rio Napo in May 2011, and nine samples were collected in Cuyabeno Wildlife Reserve in July 2011. A total of 25 samples were collected throughout the study regions.

Surface water grab sampling methodologies outlined in Florida Department of Environmental Protection Field Sampling Standard Operating Procedures were employed (FDEP FS2100) and EPA analyses were used (EPA 245.1 1994, EPA 200.7 1994, EPA 8270 2007, EPA 8260 1996, and FL-PRO 1995). The contaminants of interest were PAHs, VOCs, TPHs, and the heavy metals arsenic, lead, and mercury.

Each EPA approved method has a legally identifiable container, preservative, and holding time. The required containers for each analysis are an amber glass 1 L container with 2 mL of 1:1 H_2SO_4 for EPA 8270 and FL-PRO, two 40 mL vials with 1 mL of HCl and zero headspace for EPA 8260, and a 125 mL plastic polyethylene container with 1 mL of HNO_3 for EPA 245.1 and EPA 200.7. The holding time for each analysis is as follows: EPA 8270 and FL-PRO – 7 days to extraction, 40 days to analysis, EPA 8260 – 14 days to analysis, EPA 245.1 – 28 days to analysis, and EPA 200.7 – 6 months to analysis. Samples are also legally required to be preserved thermally at 4 degrees Celsius after collection and prior to analysis to prevent microbial degradation (FDEP FS1000, Table FS1000-4).

Water sample kits were assembled prior to each expedition. Each kit contained 1 amber glass 1 L bottle, 2 glass 40 mL vials, and 1 polyethylene 125 mL bottle. Ten kits were taken into the field during each expedition; they were placed in 2 large coolers along with labels, permanent markers, chain of custody documents, and packing material

to prevent breakage. Coolers were packed and secured with duct tape and flown to Ecuador. In addition, a butane refrigerator was purchased and transported to Ecuador in order to meet the thermal preservation requirement for samples.

Each sample was labeled with "WAT" and numbers 1 – 25, and stored in a cooler in bubble wrap in the field. The following information was recorded with each sample: time, date, GPS coordinates, and the local name of the tributary or lagoon where it was collected. At the end of each expedition, the samples were packed securely in the coolers using bubble wrap, dirty clothes, and duct tape to prevent breakage during the flight back to the Florida.

The samples were transported to Florida Spectrum Environmental Services, Inc. (FSES), a NELAC and FDOH certified (E86006) environmental laboratory. They were analyzed according the EPA methods 8270, 8260, FL-PRO, 245.1, and 200.7 by certified QC chemists. See literature cited for reference method analytical procedures.

DATA ANALYSIS

Results from the chemical analyses were compared to the results of previous water analyses conducted in eastern Ecuador in 1993 (PAHs and VOCs) and in 1998 (TPH). The concentrations of analytes in each study region (Yasuni [1], Lagartococha [2], and Cuyabeno [3]) and in past studies were compared statistically using the program JMP. Data were tested for homoscedasticity (equal variance) with Levene's test. A Mann-Whitney U (non-parametric t-test) was used to compare analytics from previous years and this study. Holding time differences were also compared statistically between the study regions using a one-way ANOVA analysis with post-hoc Tukey-Kramer HSD pair-wise testing.

RESULTS

A map of the eastern Sucumbios and Orellana provinces in Ecuador is shown in Figure 35. Light pink represents the protected areas Yasuni National Park and Cuyabeno Wildlife Reserve (see Figure 4 for reference). Water samples are shown as green dots and oil wells are shown as red and black triangles. The yellow line on the left of the map is a portion of the SOTE. Table 8 presents the GPS coordinates, date, and time of each water sample collected during the study.

The analytes, method detection limits [MDL], practical quantitation limit [PQLs], and units for arsenic, lead, mercury, and PAHs are listed in Table 9 and VOCs are listed in Table 10. MDL is the statistical method detection limit as determined by the analysis of seven replicates of a low level standard followed by the calculation of the standard deviation which is multiplied by the 99% confidence interval t statistic for 6 degrees of freedom. PQL refers to the statistical practical quantitation limit calculated by multiplying the MDL times three.

Tables 11-13 present the analytical results for heavy metals, PAHs, VOCs, and TPHs for each sample within each study area. The reported methods, analytes, units, and sensitivities for the CESR 1994 study are summarized in Table 14. Tables 15 and 16 present the analytical results from the studies in 1993 and 1998.

Figure 35: Map of Water Samples, Oil Wells, Pipelines, and Protected Areas

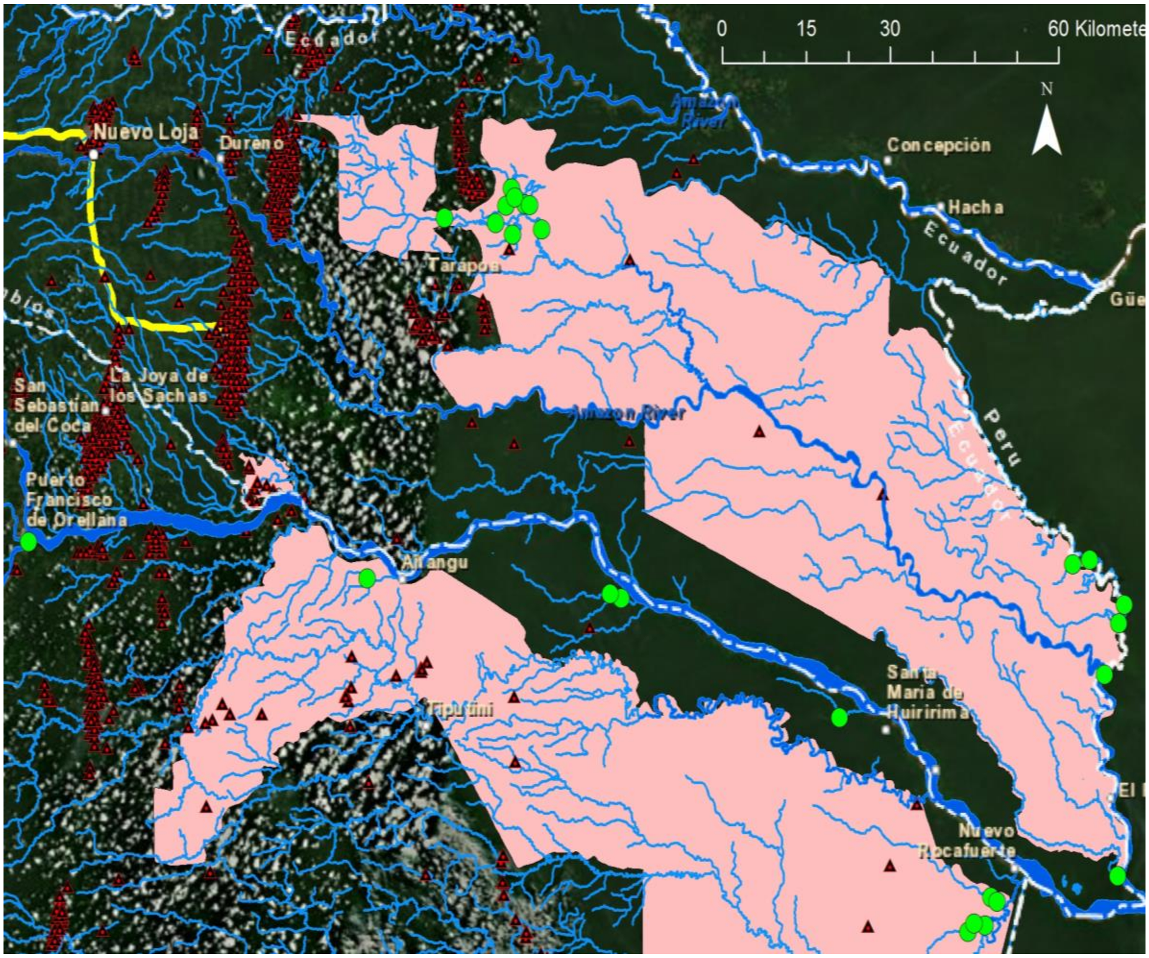


Table 8: Water Samples Collected in Yasuni National Park, Lagartococha, and Cuyabeno Wildlife Reserve

	Sample ID	Date	Time	Latitude	Longitude	Altitude
Yasuni (Study Area 1)	Tambucocha Wat-1	06-MAR-11	12:10:51	S0.96104	W75.44084	184 m
	Tambucocha Wat-2	06-MAR-11	18:08:40	S0.96719	W75.43126	188 m
	Jatuncocha Wat-3	07-MAR-11	10:43:14	S0.99915	W75.45065	175 m
	Jatuncocha Wat-4	07-MAR-11	14:30:00	S1.00860	W75.47740	183 m
	Jatuncocha Wat-5	07-MAR-11	16:24:46	S0.99723	W75.46765	179 m
	Huiririma Wat-6	08-MAR-11	13:34:02	S0.71501	W75.68322	198 m
	Yuturi Canyo Wat-7	09-MAR-11	12:38:43	S0.55114	W76.03305	199 m
	Cadiyuturi Wat-8	09-MAR-11	14:01:54	S0.54532	W76.05103	203 m
	Anangu Wat-9	10-MAR-11	12:37:35	S0.52402	W76.43957	228 m
Lagartococha (Study Area 2)	Piuri Laguna Wat-10	24-MAY-11	13:27:33	S0.50450	W75.30944	190 m
	Urcococha Wat-11	24-MAY-11	16:02:26	S0.49855	W75.28239	186 m
	Yarinacocha Wat-12	25-MAY-11	10:51:40	S0.55987	W75.22716	189 m
	Lagartococha Wat-13	25-MAY-11	17:40:20	S0.65629	W75.25915	173 m
	Clavococha Wat-14	26-MAY-11	11:01:26	S0.58606	W75.23585	189 m
	Rio Cocaya Wat-15	28-MAY-11	16:23:19	S0.93222	W75.23743	188 m
	Rio Napo Wat-16	30-MAY-11	10:54:34	S0.47386	W76.98232	239 m
Cuyabeno (Study Area 3)	Wat-17	11-JUL-11	9:20:39	N0.00979	W76.20848	222 m
	Wat-18	11-JUL-11	12:19:18	S0.01215	W76.18657	220 m
	Wat-19	12-JUL-11	9:15:13	S0.01322	W76.21747	219 m
	Wat-20	12-JUL-11	11:13:31	S0.00214	W76.20355	224 m
	Wat-21	13-JUL-11	9:31:49	S0.05306	W76.20716	220 m
	Wat-22	13-JUL-11	12:55:11	S0.04618	W76.16073	241 m
	Wat-23	14-JUL-11	14:26:34	S0.01272	W76.17993	230 m
	Wat-24	16-JUL-11	9:58:54	S0.03705	W76.23435	210 m
	Wat-25	16-JUL-11	11:26:10	S0.03028	W76.31611	205 m

Table 9: Chemical Analysis Parameters: Heavy Metals, PAHs, and TPHs

	Analyte	EPA Method	MDL	PQL	UNITS
Heavy Metals	**Arsenic**	200.7	0.001181	0.0035	mg/L
	Lead	200.7	0.001121	0.0034	mg/L
	Mercury	245.1	0.070616	0.2118	ug/L
PAHs	**1-Methylnapthalene**	8270D	0.040	0.12	ug/L
	2-Methylnapthalene	8270D	0.072	0.216	ug/L
	Acenaphthene	8270D	0.016	0.048	ug/L
	Acenaphthylene	8270D	0.012	0.036	ug/L
	Anthracene	8270D	0.012	0.036	ug/L
	Benzo(a)anthracene	8270D	0.008	0.024	ug/L
	Benzo(a)pyrene	8270D	0.012	0.036	ug/L
	Benzo(b)fluoranthene	8270D	0.016	0.048	ug/L
	Benzo(ghi)perylene	8270D	0.014	0.042	ug/L
	Benzo(k)fluoranthene	8270D	0.006	0.018	ug/L
	Chrysene	8270D	0.008	0.024	ug/L
	Dibenzo(a,h)anthracene	8270D	0.008	0.024	ug/L
	Fluoranthene	8270D	0.008	0.024	ug/L
	Fluorene	8270D	0.016	0.048	ug/L
	Indeno(1,2,3-cd)pyrene	8270D	0.022	0.066	ug/L
	Napthalene	8270D	0.054	0.162	ug/L
	Phenanthrene	8270D	0.026	0.078	ug/L
	Pyrene	8270D	0.022	0.066	ug/L
Petroleum	**Total Petroleum Residual Organics**	FL-PRO	0.14	0.42	mg/L

Table 10: Chemical Analysis Parameters: VOCs

	Analyte	EPA Method	MDL	PQL	Units
VOCs	**1,1,1,2-Tetrachloroethane**	5030/8260B	0.15	0.45	ug/L
	1,1,1-Trichloroethane	5030/8260B	0.67	2.01	ug/L
	1,1,2,2-Tetrachloroethane	5030/8260B	0.14	0.42	ug/L
	1,1,2-Trichloroethane	5030/8260B	0.46	1.38	ug/L
	1,1-Dichloroethane	5030/8260B	0.19	0.57	ug/L
	1,1-Dichloroethene	5030/8260B	0.42	1.26	ug/L
	1,1-Dichloropropene	5030/8260B	0.65	1.95	ug/L
	1,2,3-Trichlorobenzene	5030/8260B	0.28	0.84	ug/L
	1,2,3-Trichloropropane	5030/8260B	0.22	0.66	ug/L
	1,2,4-Trichlorobenzene	5030/8260B	0.23	0.69	ug/L
	1,2,4-Trimethylbenzene	5030/8260B	0.38	1.14	ug/L
	1,2-Dibromo-3-chloropropane	5030/8260B	0.17	0.51	ug/L
	1,2-Dibromoethane (EDB)	5030/8260B	0.25	0.75	ug/L
	1,2-Dichlorobenzene	5030/8260B	0.3	0.9	ug/L
	1,2-Dichloroethane	5030/8260B	0.31	0.93	ug/L
	1,2-Dichloropropane	5030/8260B	0.46	1.38	ug/L
	1,3,5-Trimethylbenzene	5030/8260B	0.38	1.14	ug/L
	1,3-Dichlorobenzene	5030/8260B	0.4	1.2	ug/L
	1,3-Dichloropropane	5030/8260B	0.46	1.38	ug/L
	1,4-Dichlorobenzene	5030/8260B	0.39	1.17	ug/L
	2,2-Dichloropropane	5030/8260B	0.76	2.28	ug/L
	2-Butanone (Methyl Ethyl Ketone)	5030/8260B	0.56	1.68	ug/L
	2-Chloroethylvinyl ether	5030/8260B	0.76	2.28	ug/L
	2-Chlorotoluene	5030/8260B	0.38	1.14	ug/L
	4-Chlorotoluene	5030/8260B	0.33	0.99	ug/L
	Acetone	5030/8260B	1.42	4.26	ug/L
	Acrolein	5030/8260B	6.99	20.97	ug/L
	Acrylonitrile	5030/8260B	0.52	1.56	ug/L
	Benzene	5030/8260B	0.14	0.42	ug/L
	Bromobenzene	5030/8260B	0.4	1.2	ug/L
	Bromochloromethane	5030/8260B	0.21	0.63	ug/L
	Bromodichloromethane	5030/8260B	0.52	1.56	ug/L
	Bromoform	5030/8260B	0.16	0.48	ug/L
	Bromomethane (Methyl Bromide)	5030/8260B	0.6	1.8	ug/L
	Carbon Tetrachloride	5030/8260B	0.81	2.43	ug/L
	Chlorobenzene	5030/8260B	0.34	1.02	ug/L

	Analyte	EPA Method	MDL	PQL	Units
	Chloroethane	5030/8260B	0.47	1.41	ug/L
	Chloroform	5030/8260B	0.27	0.81	ug/L
	Chloromethane (Methyl Chloride)	5030/8260B	0.88	2.64	ug/L
	cis-1,2-Dichloroethene	5030/8260B	0.17	0.51	ug/L
	cis-1,3-Dichloropropene	5030/8260B	0.41	1.23	ug/L
	Dibromochloromethane	5030/8260B	0.3	0.9	ug/L
	Dibromomethane	5030/8260B	0.37	1.11	ug/L
	Dichlorodifluoromethane	5030/8260B	1.06	3.18	ug/L
	Ethyl Benzene	5030/8260B	0.42	1.26	ug/L
	Hexachlorobutadiene	5030/8260B	0.47	1.41	ug/L
	Isopropylbenzene	5030/8260B	0.38	1.14	ug/L
	m,p-Xylene	5030/8260B	0.8	2.4	ug/L
	Methylene Chloride	5030/8260B	0.99	2.97	ug/L
	Methyl-tert-butyl ether	5030/8260B	0.55	1.65	ug/L
	Naphthalene	5030/8260B	0.24	0.72	ug/L
	n-Butyl Benzene	5030/8260B	0.34	1.02	ug/L
	n-Propylbenzene	5030/8260B	0.39	1.17	ug/L
	o-Xylene	5030/8260B	0.32	0.96	ug/L
	p-Isopropyltoluene	5030/8260B	0.41	1.23	ug/L
	sec-Butyl Benzene	5030/8260B	0.45	1.35	ug/L
	Styrene	5030/8260B	0.31	0.93	ug/L
	tert-Butylbenzene	5030/8260B	0.4	1.2	ug/L
	Tetrachloroethene	5030/8260B	0.42	1.26	ug/L
	Toluene	5030/8260B	0.31	0.93	ug/L
	trans-1,2-Dichloroethene	5030/8260B	0.21	0.63	ug/L
	trans-1,3-Dichloropropene	5030/8260B	0.28	0.84	ug/L
	Trichloroethene	5030/8260B	0.34	1.02	ug/L
	Trichlorofluoromethane	5030/8260B	0.48	1.44	ug/L
	Vinyl chloride	5030/8260B	0.79	2.37	ug/L

Table 11: Concentrations of Contaminants Measured in Yasuni National Park
"U" – analyte not detected above the MDL.

#	Site Name	Arsenic (mg/L)	Lead (mg/L)	Mercury (ug/L)	PAHs (ug/L)	TPHs (mg/L)	VOCs (ug/L)
1	Tambucocha	U	U	U	U	0.393	U
2	Tambucocha	U	U	U	U	0.435	U
3	Jatuncocha	U	U	U	U	0.438	U
4	Jatuncocha	U	U	U	U	0.399	U
5	Jatuncocha	U	U	U	U	0.382	U
6	Huiririma	U	U	U	U	U	U
7	Yuturi Canyo	U	U	U	U	0.419	U
8	Cadi Yuturi	U	U	U	U	U	U
9	Añangu	U	U	U	U	0.389	U

Table 11 shows that TPHs were the only contaminants detected in Yasuni.

Table 12: Concentrations of Contaminants Measured in Lagartococha
"U" – analyte not detected above the MDL.

#	Site Name	Arsenic (mg/L)	Lead (mg/L)	Mercury (ug/L)	PAHs (ug/L)	TPHs (mg/L)	VOCs (ug/L)
10	Piuri Laguna	U	U	U	U	U	U
11	Urcococha	U	U	U	U	U	U
12	Yarinacocha	U	U	U	U	U	U
13	Lagartococha	U	U	U	U	U	U
14	Clavococha	U	U	U	U	U	U
15	Rio Cocaya	U	U	U	U	U	U
16	Rio Napo	U	0.002	U	U	U	U

Table 12 shows that no TPHs were detected in Lagartococha. A small amount of lead was detected in one sample.

Table 13: Concentrations of Contaminants Measured in Cuyabeno Wildlife Reserve
"U" – analyte not detected above the MDL.
N/A – Sample 23 not analyzed for PAHs/TPHs due to broken bottle.

#	Site Name	Arsenic (mg/L)	Lead (mg/L)	Mercury (ug/L)	PAHs (ug/L)	TPHs (mg/L)	VOCs (ug/L)
17	-	U	U	U	U	U	U
18	-	U	U	U	U	U	U
19	-	U	U	U	U	U	U
20	-	U	U	U	U	U	U
21	-	U	U	U	U	U	U
22	-	U	U	U	U	U	U
23	-	U	U	U	N/A	N/A	U
24	-	U	U	U	U	U	U
25	-	U	U	U	U	U	U

Table 13 shows that no contaminants of concern were detected in Cuyabeno.

Table 14: Historical Chemical Analysis Parameters from CESR 1994

Analyte	EPA Method	MDL	PQL	Units
Total PAHs	EPA 8270	Not Reported	10	ng/L
Benzene	EPA 8020	Not Reported	Not Reported	ug/L
Toluene	EPA 8020	Not Reported	Not Reported	ug/L
Ethyl Benzene	EPA 8020	Not Reported	Not Reported	ug/L
Total Xylenes	EPA 8020	Not Reported	Not Reported	ug/L

Table 14: This table shows the chemical analysis methods, analytes, MDLs, PQLs, and units for PAHs and VOCs measured by CESR 1994. Sebastian *et al.* 2001 did not report methods or sensitivities for TPHs.

Table 15: Concentrations of Contaminants Measured by CESR 1994

#	Site Name	Total PAH (ng/L), 1993	VOCs (ug/L), 1993
1	Fanny, City	46,423	186.2
1a	Duplicate	46,523	239
2	Shushufindi N	263,119	4050
3	Shushufindi S	91,300	2540
4	Sacas, Central	405,634	2676
5	Dayuma, Lagoon	49,931	U
6	San Pablo	233.27	U
7	San Pablo, Opposite	108.08	U
8	128 km S of Coca	32.8	U
9	Shushufindi	448.62	U
10	Sachas, Pimampiro	44.23	U
11	Sachas, Black	696.09	U
12	Sachas, Beige	2,798.93	U
13	San Pablo, Texaco	55.21	U
14	Dureno River	137.46	U
15	Shushufindi River	37.22	U
16	Eno River	40.93	U
17	El Dorado River	134.26	U
18	Qinchayacu River	152.92	U
19	Dayuma, bathing pool	40.62	U
20	128 km south of Coca, former bathing pool	306.22	U
21	Shushufindi North Station stream, former bathing pool	1,486.53	U
22	Sachas stream, former bathing site	129.35	U

Table 15: The concentrations of PAHs and VOCs measured in 1993 (CESR 1994) in surface water samples are summarized in Table 15. "U" means the analyte was not detected above the MDL.

Table 16: Concentrations of Contaminants Measured by Sebastian *et al.* 2001

#	Site Name 1998	TPHs 1998 (mg/L)
1	Parker	0.53
2	Huamayacu	1.444
3	Basura	2.883
4	Iniap	0.097

Table 16: The concentrations of TPHs measured by Sebastian *et al.* in 1998 are shown in Table 16.

There were noteworthy differences in TPH concentration among the three study regions (See Tables 11-13 and Table 17). TPH was detected in study area 1 and they were not detected in study areas 2 and 3 indicating a potential source of contamination in the Yasuni study region.

Table 17: Mean TPH by Study Region

Study Region	Mean TPH Concentration (mg/L)
1 (Yasuni, n=9)	0.317222
2 (Lagartococha, n=7)	0.00
3 (Cuyabeno, n=8)	0.00

Table 18: Holding Times for PAH, TPH, and VOC Analyses by Study Region
Note: ~indicates recommended FDEP holding time exceeded; metals were excluded.

#	Site Name	PAH Holding Time (days)	TPH Holding Time (days)	VOC Holding Time (days)
1	Tambucocha	9~	10~	9
2	Tambucocha	9~	10~	9
3	Jatuncocha	8~	9~	8
4	Jatuncocha	8~	9~	8
5	Jatuncocha	8~	9~	8
6	Huiririma	7	8~	7
7	Yuturi Canyo	6	7	6
8	Cadi Yuturi	6	7	6
9	Añangu	5	6	5
Average Holding Time Study Area 1		7.3	8.3	7.3
10	Piuri Laguna	9~	9~	17~
11	Urcococha	9~	9~	17~
12	Yarinacocha	8~	8~	16~
13	Lagartococha	8~	8~	16~
14	Clavococha	7	7	15~
15	Rio Cocaya	6	6	14
16	Rio Napo	4	4	11
Average Holding Time Study Area 2		7.3	7.3	15.1
17	-	15~	12~	13
18	-	15~	12~	13
19	-	14~	11~	12
20	-	13~	10~	11
21	-	13~	10~	11
22	-	12~	9~	10
23	-	N/A	N/A	9
24	-	11~	8~	9
25	-	10~	7	8
Average Holding Time Study Area 3		12.9	9.9	10.7

Table 18: This table shows the length of time that elapsed between sampling date and analysis date for each analysis excluding metals.

A one-way ANOVA and Tukey-Kramer Honest Significant Difference (HSD) were used to test for TPH sample holding time differences among the three study regions and the results are depicted in Figure 36, Table 19, and Table 20. Holding times for TPH differed significantly across the three study areas, $F(2, 21) = 4.6202$, p=0.0217. Tukey-Kramer results are presented in the least significant difference (LSD) threshold matrix and the mean TPH holding time for study area 3 ($\bar{x}$=9.88, 95%CI [8.65, 11.1]) was significantly different than study area 2 ($\bar{x}$=7.29, 95%CI [5.98, 8.60]), p=0.0179. The TPH holding time for study area 1 ($\bar{x}$=8.33, 95%CI [7.18, 9.49] was not significantly different from 2 or 3 at p=0.4394, 0.1623 indicating that holding time differences did not influence measurement of TPH.

Figure 36: TPH Holding Time (days) By Study Region

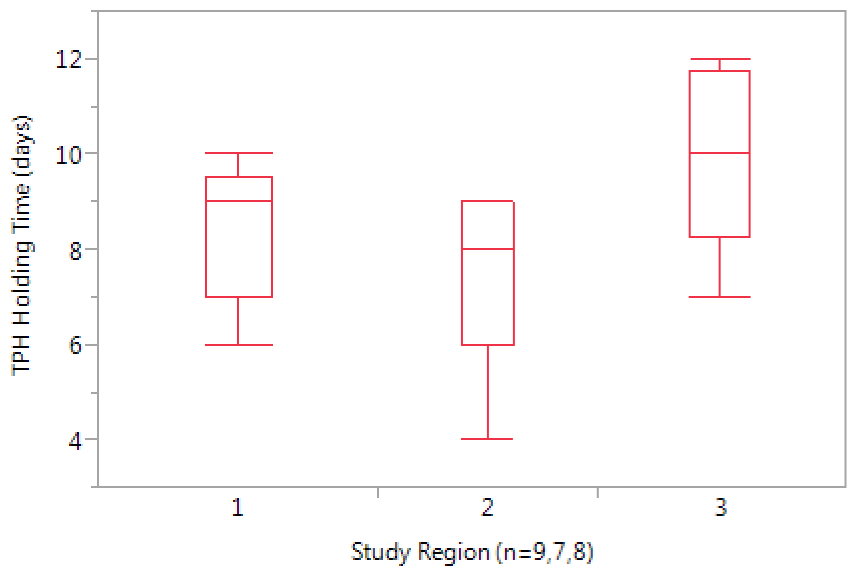


Figure 36 shows red box plots which represent the minimum, 25% quantile, median, 75% quantile, and maximum for each study region's TPH sample holding times.

Table 19: Results of One-way ANOVA Statistical Analysis of Variance for TPH Holding Time by Study Region

Source	DF	Sum of Squares	Mean Square	F Ratio	Significance
Between Study Areas	2	25.654762	12.8274	4.6202	0.0217*
Within Study Areas	21	58.303571	2.7764		
Corrected Total	23	83.958333			

Table 20: LSD Threshold Matrix for TPH Holding Time by Study Region

Abs(Dif)-HSD	3	1	2
3	-2.0999	-0.4991	0.4156
1	-0.4991	-1.9798	-1.0689
2	0.4156	-1.0689	-2.2449

Positive values show pairs of means that are significantly different.

Levene's test for equal variances and Mann-Whitney U test were used to compare the TPH concentrations from this study to the concentrations from 1998 and the results are depicted in Figure 37, Table 21, and Table 22. The null hypothesis that variances (standard deviations) are equal was rejected with Levene's test, $F(1) = 39.9975$, $p<0.0001$, and a Mann-Whitney U test assuming unequal variance was used. The results showed that there was a significant difference between the TPH concentration in 1998($\bar{x}$=1.2385, SD=1.23175) and the TPH concentration in 2011($\bar{x}$= 0.11896, SD=0.18973); z =3.01710, p=0.0026.

Figure 37: Concentration of TPHs (C8-C40) mg/L by Analysis Date

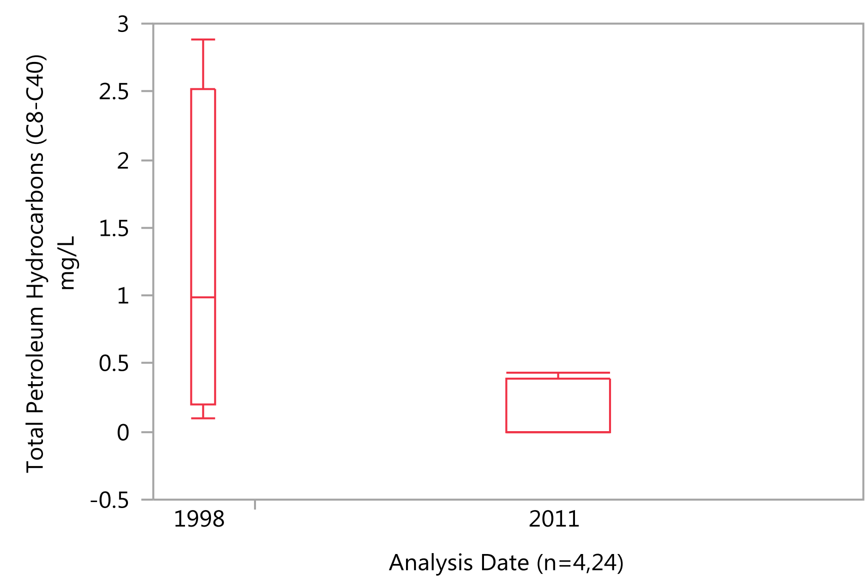


Figure 37 shows red box plots which represent the minimum, 25% quantile, median, 75% quantile, and maximum for 1998 and 2011 TPH concentrations.

Table 21: Levene's Test for Equal Variances

Source	DF	F Ratio	Significance
Between Study Years	1	39.9975	<0.0001*

Table 22: Non-Parametric Mann-Whitney Test with Unequal Variances

Year	Mean	SD	z-Stat	Prob>|z|
1998	1.2385	1.23175	3.01710	0.0026
2011	0.11896	0.18973		

DISCUSSION

Lead was detected in one sample from study area 2. The sample from Rio Napo contained 2 ug/L lead (See Table 23). The Maximum Contaminant Level (MCL) for Lead in drinking water is 15 ug/L for the US EPA, thus, the level of lead measured in Rio Napo was not of concern. No historical data could be located for this analyte in the study region. This was surprising as I expected more heavy metals to be present in the rivers and lagoons surveyed.

Wastewater runoff from ponds used to hold formation water from exploratory petroleum can supply considerable amounts of heavy metals to lakes and rivers, as previously stated, both in dissolved form and attached to particulates (Stewart 1994, Garabino *et al.* 1995). The lack of measurable levels of heavy metals could be attributed to them moving quickly downstream with the currents, being adsorbed to the surfaces of river sediments, or bioaccumulating in the bodies of organisms that inhabit the Amazon River. Suspended sediments or metallic solids can aggregate to form particles denser than water, then settle into river-bottom sediments (Garabino *et al.* 1995). The samples in this study were taken from only the surface. Heavy metal concentrations in the study area could have a differential vertical profile in the water column with more dissolved species present closer to the bottom sediments. The sampling method may have misrepresented contamination by heavy metals in the study areas.

Table 23: Summary Table of Heavy Metals and TPHs Measured and Historical TPH Data (Sebastian *et al.* 2001)
Note: * indicates sample impacted by petroleum operations; “U” means analyte not detected above the MDL.

	#	Site Name	As (mg/L)	Pb (ug/L)	Hg (ug/L)	TPHs (mg/L)	Historical Data		
Study Area 1	1	Tambucocha*	U	U	U	0.393	# 1998	Site Name 1998	TPHs 1998 (mg/L)
	2	Tambucocha*	U	U	U	0.435	1	Parker*	0.53
	3	Jatuncocha*	U	U	U	0.438	2	Huamayacu*	1.444
	4	Jatuncocha*	U	U	U	0.399	3	Basura*	2.883
	5	Jatuncocha*	U	U	U	0.382	4	Iniap*	0.097
	6	Huiririma*	U	U	U	U			
	7	Yuturi Canyo*	U	U	U	0.419			
	8	Cadi Yuturi*	U	U	U	U			
	9	Añangu*	U	U	U	0.389			
Study Area 2	10	Piuri Laguna	U	U	U	U			
	11	Urcococha	U	U	U	U			
	12	Yarinacocha	U	U	U	U			
	13	Lagartococha	U	U	U	U			
	14	Clavococha	U	U	U	U			
	15	Rio Cocaya	U	U	U	U			
	16	Rio Napo*	U	2.000	U	U			
Study Area 3	17	-*	U	U	U	U			
	18	-*	U	U	U	U			
	19	-*	U	U	U	U			
	20	-*	U	U	U	U			
	21	-*	U	U	U	U			
	22	-*	U	U	U	U			
	23	-*	U	U	U	N/A			
	24	-*	U	U	U	U			
	25	-*	U	U	U	U			
Regional TPH				TPH Study Area 1 > 2 and 3		Historical TPH Mann-Whitney U Test			*z*= 3.01710 P= 0.0026

TPHs were detected in samples 1-5, 7, and 9 from the Yasuni National Park study and the results are listed in Table 23. The MCL for hydrocarbons in drinking water is 10 ug/L as regulated by the European Community laws (Sebastian *et al.* 2001). There are no specific TPH MCLs for drinking water or surface water regulated by the EPA or FDEP. Instead, individual constituents of TPH (PAHs, VOCs, etc.) are regulated with MCLs. None of these were detected in the samples.

TPHs were detected in study area 1 (Yasuni) and were not detected in study areas 2 and 3 (Lagartococha and Cuyabeno) (See Table 17 and Table 23). Since no contamination by TPH was measured in study areas 2 and 3 they were essentially "clean". The results of the chemical analysis for TPH support the hypothesis that toxic contaminants do exist in study area 1 and indicate a potential source of contamination in Yasuni National Park. In addition, these results support the hypothesis that levels of contamination vary spatially. It is possible that the concentrations measured in the samples from our study were biased low due to the lack of thermal or chemical preservation, but high levels of contaminants would still be detectable. Sample holding time differences do not explain observed differences in TPH (See Figure 36, Tables 18-20).

It is not clear if the variation in contamination observed between study regions is a function of oil activities. A study in 1998 found no water contamination by TPH in areas lacking oil activities (Sebastian and Hurtig 2004). Figure 35 shows water samples, oil pipelines, and oil wells. There are numerous oil wells near and within Yasuni National Park and Cuyabeno Wildlife Reserve and a large pipeline close to Cuyabeno Wildlife Reserve. However, there are no oil wells or pipelines close in proximity to the Lagartococha study area. I expected not to find any contamination near Lagartococha, but was surprised to find no contamination in Cuyabeno Wildlife Reserve because it is close to a major oil pipeline and numerous oil wells and there was an oil spill there in 1995 (Marmontel 2008) The last major oil spill in Yasuni was in 2008 (Finer *et al.* 2008). This study was performed much closer temporally to the oil spill reported in Yasuni (2008) than to the spill reported in Cuyabeno (1995). The lack of measurable concentrations of contaminants in the Cuyabeno region could be due to biodegradation, bioaccumulation, adsorption of contaminants to river sediments, or contaminants moving downstream. Further investigation is needed test other hypotheses.

Table 22 demonstrates that the concentration of TPHs was significantly different than the levels measured in 1998. A Mann-Whitney U test assuming unequal variances was used to compare the levels of TPH measured in 1998 to those measured in this study. The result of the analysis was a significant difference between the two studies ($p=0.0026$)

(See Figure 37, Tables 21-22). These results support the hypothesis that the level of petroleum hydrocarbons in the Ecuadorian Amazon may have decreased since last measured by Sebastian *et al.* 2001. However, there are many variables and further research is necessary to test this hypothesis.

Table 24: Summary of PAHs and VOCs Measured and Historical PAH and VOC Data (CESR 1994)

Note: * indicates sample impacted by petroleum operations; "U" means analyte not detected above the MDL.

	#	Site Name	PAHs (ng/L)	VOCs (ug/L)	Historical Data			
Study Area 1	1	Tambucocha*	U	U	# 1994	Site Name 1994	PAHs (ng/L)	VOCs (ug/L)
	2	Tambucocha*	U	U	1	Fanny, City*	46,423	186.2
	3	Jatuncocha*	U	U	1a	Duplicate*	46,523	239
	4	Jatuncocha*	U	U	2	Shushufindi N*	263,119	4050
	5	Jatuncocha*	U	U	3	Shushufindi S*	91,300	2540
	6	Huiririma*	U	U	4	Sacas, Central*	405,634	2676
	7	Yuturi Canyo*	U	U	5	Dayuma, Lagoon*	49,931	U
	8	Cadi Yuturi*	U	U	6	San Pablo*	233.27	U
	9	Añangu*	U	U	7	San Pablo, Opposite*	108.08	U
Study Area 2	10	Piuri Laguna	U	U	8	128 km S of Coca	32.8	U
	11	Urcococha	U	U	9	Shushufindi*	448.62	U
	12	Yarinacocha	U	U	10	Sachas, Pimampiro*	44.23	U
	13	Lagartococha	U	U	11	Sachas, Black*	696.09	U
	14	Clavococha	U	U	12	Sachas, Beige*	2,798.93	U
	15	Rio Cocaya	U	U	13	San Pablo, Texaco*	55.21	U
	16	Rio Napo*	U	U	14	Dureno*	137.46	U
Study Area 3	17	-*	U	U	15	Shushufindi River	37.22	U
	18	-*	U	U	16	Eno River	40.93	U
	19	-*	U	U	17	El Dorado River	134.26	U
	20	-*	U	U	18	Qinchayacu River*	252.92	U
	21	-*	U	U	19	Dayuma Pool*	40.62	U
	22	-*	U	U	20	Coca*	306.22	U
	23	-*	U	U	21	Shushufindi*	1,486.53	U
	24	-*	U	U	22	Sachas*	12.35	U
	25	-*	U	U				

Table 24 shows that surface water samples obtained during this study contained lower amounts of PAHs than those in the 1994 CESR study. These findings support the hypothesis that the concentration of toxic PAHs in the Ecuadorian Amazon differed since last studied by CESR in 1994. The water samples from the CESR 1994 study were taken from production water sites (oil production wells and open wastewater ponds), drinking water sites, and surface water sites. The research presented in this thesis includes samples

from surface water sites therefore comparisons were made using only data from comparable sources (Samples 14-22 from CESR 1994). Concentrations of PAHs at production water sites (samples 1-5 CESR 1994) were much higher than at surface water sites. This indicates that when production water disperses in the environment the concentrations are diluted or absorbed by natural processes.

Table 24 demonstrates that the concentrations of VOCs in surface water sites measured in the CESR (1994) study did not differ from those measured in this study. CESR (1994) reported high concentrations of VOCs in petroleum production waters. VOCs readily volatilize to the atmosphere because of their high vapor pressure, thus, it is logical that they are present at high concentrations near oil production sites but are dilute in ambient waters. The data from this study support the hypothesis that VOC concentrations in ambient water did not differ since last studied by CESR in 1994.

It has been reported that exposure to crude oil is not limited to the immediate area of contamination. When discharged into the environment, heavier, less volatile constituents (PAHs, Heavy Metals, and TPHs) tend to sink and adsorb into sediments from which they may either enter the food chain through benthic organisms or repeatedly contaminate the water column. Lighter compounds such as VOCs may evaporate in a matter of hours and be deposited via rain or air to locations far from the source (CESR 1994). Thus, toxic chemicals found in crude oil may still be present in the environment even if not detected in water samples. Sediments and plants can be good indicators of environmental pollution (Goncalves, Boaventura, and Mouvet 1992) and contaminants can build up in the tissues of marine mammals (Belanger and Wittnich 2008). Going forward, the analysis of sediment samples, plant samples, air samples, and biological tissue samples from marine mammals for constituents of crude oil will provide more conclusive evidence about the extent of environmental contamination in the Ecuadorian Amazon.

PROBLEMS ENCOUNTERED

All the appropriate bottles were used; however, mineral preservatives as prescribed by each EPA method and FDEP regulation were not immediately added to

samples in the field due to transportation restrictions. The analysis of heavy metals by EPA 200.7 and EPA 245.1 is not affected if samples are preserved chemically in the laboratory. The primary reason the samples are preserved with HNO3 is because the metals adsorb to the plastic sample bottle. Microbes do not affect concentrations, and once the samples are preserved in the laboratory, the concentration in the sample is still representative of the source. However, the concentrations of PAHs, VOCs, and TPHs could have been biased low if microbes partially degraded the analytes or analytes became unstable due to the lack of chemical preservation.

In addition to not meeting the chemical preservation requirement, thermal preservation could not be performed. A butane operated refrigerator was purchased to be used in the field for thermal preservation but it was found to be inoperative when tested in the field. Thermal preservation does not affect metals analyses, again, because heavy metals are not subject to microbial degradation. The analyses of VOCs, TPH, and PAHs could have been biased low if microbes metabolized a portion of the target analytes or chemical reactions occurred due to the warm temperature. High concentrations of contaminants are still measurable even if not chemically or thermally preserved.

Analytes are least likely to have degraded when analyzed within the holding times. Due to the lengthy transport of samples to the laboratory some of the samples were analyzed outside the recommendations. Due to the length of time required to travel to the study areas, perform the study, and then transport samples back to the United States, the holding times simply could not be met for all samples (See Table 18). This also could have contributed to low bias because holding times are set based on the length of time target analytes are stable in matrices after sampling. The holding times for mercury, lead, and arsenic were a non-issue because they are much longer than the organic analytes. It’s possible that PAHs are stable for up to 22 days according to a recent holding time study (Gallotta *et al.* 2010). VOCs have a high vapor pressure and decrease rapidly with time as they vaporize. High concentrations of contaminants would likely still be detectable outside of required holding times.

The only analyte with a short holding time detected in samples was TPH. Differences in holding times for TPH samples between study areas were analyzed using

one-way ANOVA and post-hoc Tukey-Kramer HSD. A one-way ANOVA analysis of study region holding time showed that there was significant difference between study regions ($p=0.0217$). However, the Tukey-Kramer HSD showed the TPH holding time for study areas 2 and 3 did not differ significantly from study area 1 (See Figure 36, Tables 19-20). Therefore, holding time differences did not account for the differences in TPH concentration between study regions.

The lagoon or tributary name was not recorded during the Cuyabeno expedition. Although no “named” location was associated with these samples, GPS coordinates were recorded for geographic and study area reference. Finally, inadequate packing material during transport caused a broken sample bottle for “Wat 23”from the Cuyabeno Wildlife Reserve expedition. Therefore, the PAHs and TPHs in that sample were not measured.

CONCLUSIONS

The results of this study support the hypothesis that some toxic contaminants exist in study area 1, which is a part of the Amazonian manatee habitat in Ecuador. Lead, arsenic, mercury, PAHs, and VOCs were not detected in the surface waters of the study region. High TPH levels were measured in 7 samples from Yasuni National Park. TPHs were detected in Yasuni National Park and were not detected in Lagartococha and Cuyabeno. These data support the conclusion that toxic contaminants vary spatially from region to region. There was no evidence that TPHs were higher near production wells and pipelines. TPHs were detected only in the study region with a recent oil spill.

The data in this study support the hypothesis that levels of TPH differed since last studied by Sebastian *et al.* (2001), the levels of PAHs differed, and the concentration of VOCs did not differ from CESR (1994). It is recommended that a dedicated study be performed to develop a protocol for monitoring persistent oil contaminants in the Ecuadorian Amazon. In the future, studies should focus on sites near contaminated production water or wastewater discharges and improving the sampling process to better represent the study area. Evaluations should include sampling water at depth, sediment, plants, air, and biological tissue to more extensively evaluate the environmental contamination; meeting thermal and chemical preservation requirements to eliminate the

possibility of low bias; and evaluating how contamination disperses or degrades in the environment by investigating a limited study area over time.

CHAPTER IV

EXECUTIVE SUMMARY

In Ecuador, the population of Amazonian manatees is critically endangered due to a multitude of anthropogenic threats including hunting, habitat destruction, and incidental mortality from gillnets (Marmontel 2008). However, due to the species' elusive nature, the current status of the population should be classified as unknown – data deficient. Population studies are rare, do not provide valid population estimates, and therefore do not allow for long-term assessment of changes (Timm *et al.* 1986, Denkinger 2010). The environment makes visual surveys challenging, aquatic mammals are difficult to observe by nature, and the Amazonian manatee is a very secretive creature that spends most of its time submerged as an adaptation to hunting pressures (Marmontel 2008). Therefore, a viable method for collecting population data must be established. Side-scan sonar is a viable method for establishing and monitoring Amazonian manatee population numbers in the Ecuadorian Amazon.

Legally, Amazonian manatees are protected in Ecuador by "Resolucion No. 105" of the Ministry of the Environment (January 28, 2000) (Rios personal comm.). Thus, in order for the government to comply with legal obligations to adequately protect the species from extinction, the population status must be determined. After the establishment of viable estimates in different regions, models can be used to make decisions, to more accurately predict the trend of the population, and to concentrate conservation efforts in areas where manatees are more abundant. This could be accomplished by the establishment of protected areas where hunting and pollution laws are strictly enforced.

The destruction of Amazonian manatee habitats is largely attributable to the development of the petroleum extraction industry in the Ecuadorian Amazon. An increase in the scope and magnitude of planned hydrocarbon activity in the primary habitat is inevitable and documented contamination issues are likely to intensify (Finer *et al.* 2008). Legally, oil companies are required to regularly monitor pollution, but it has been suggested that appropriate limits are not being enforced (Sebastian and Hurting 2004). In

order to protect the environment, it is pertinent to establish a protocol to assess the extent, current levels, spatial and temporal variation, contaminated areas, and final destination of chemicals in the environment and set strictly enforced limits.

The Ecuadorian government is not providing adequate protection for the Amazonian manatee, the environment, and the people inhabiting the Amazon. It is a governmental obligation to establish and enforce laws which will protect natural resources and citizens of the nation. There are laws which intend to protect the Amazonian manatee and prevent pollution of the environment in Ecuador, but these laws are useless if they are not enforced. In summary, there are 11 major points resulting from this research project, which are important for consideration by decision makers responsible for conservation of manatees and their habitats in the eastern Ecuadorian Amazon:

1) The population of Amazonian manatees in eastern Ecuador is extant, in contrast with the Timm *et al.* (1986) prediction.
2) The results of this study demonstrate that the side-scan sonar method (Gonzalez-Socoloske *et al.* 2009) is a viable method for detecting Amazonian manatees in the Ecuadorian Amazon.
3) Manatees were encountered more often in Cuyabeno Wildlife Reserve than in Lagartococha and Yasuni. The probability of manatee detection was greater in Cuyabeno Wildlife Reserve and Lagartococha than reported in 1996-1999 (Denkinger 2010).
4) Side-scan sonar detected more manatees than previously reported by Denkinger in 2010 and Timm *et al.* 1986.
5) Side-scan sonar resulted in greater detection as a function of effort when compared to visual surveys.
6) The data from this study demonstrate that manatees were detected in an area where we assume they cannot be detected.
7) The results of this study support the hypothesis that some toxic contaminants exist in study area 1, which is a part of the Amazonian manatee habitat in Ecuador.

8) Lead, arsenic, mercury, PAHs, and VOCs were not detected in the surface waters of the study region.

9) High TPH levels were measured in 7 samples from Yasuni National Park. TPHs were detected in Yasuni National Park and were not detected in Lagartococha and Cuyabeno. These data support the conclusion that toxic contaminants vary spatially from region to region.

10) There was no evidence that TPHs were higher near production wells and pipelines. TPHs were detected only in the study region with a recent oil spill.

11) The data in this study support the hypothesis that levels of TPH differed since last studied by Sebastian *et al.* (2001), the levels of PAHs differed, and the concentration of VOCs did not differ from CESR (1994).

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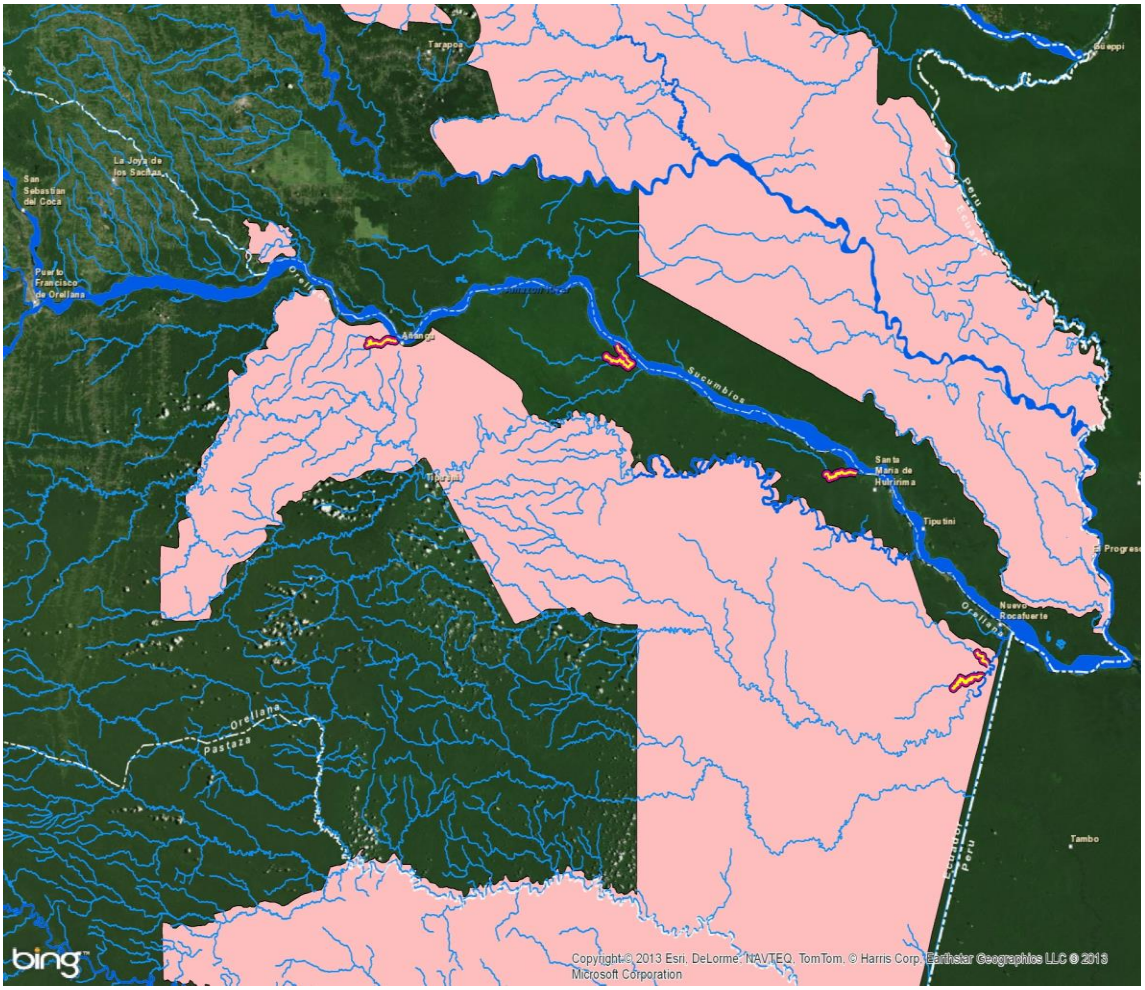
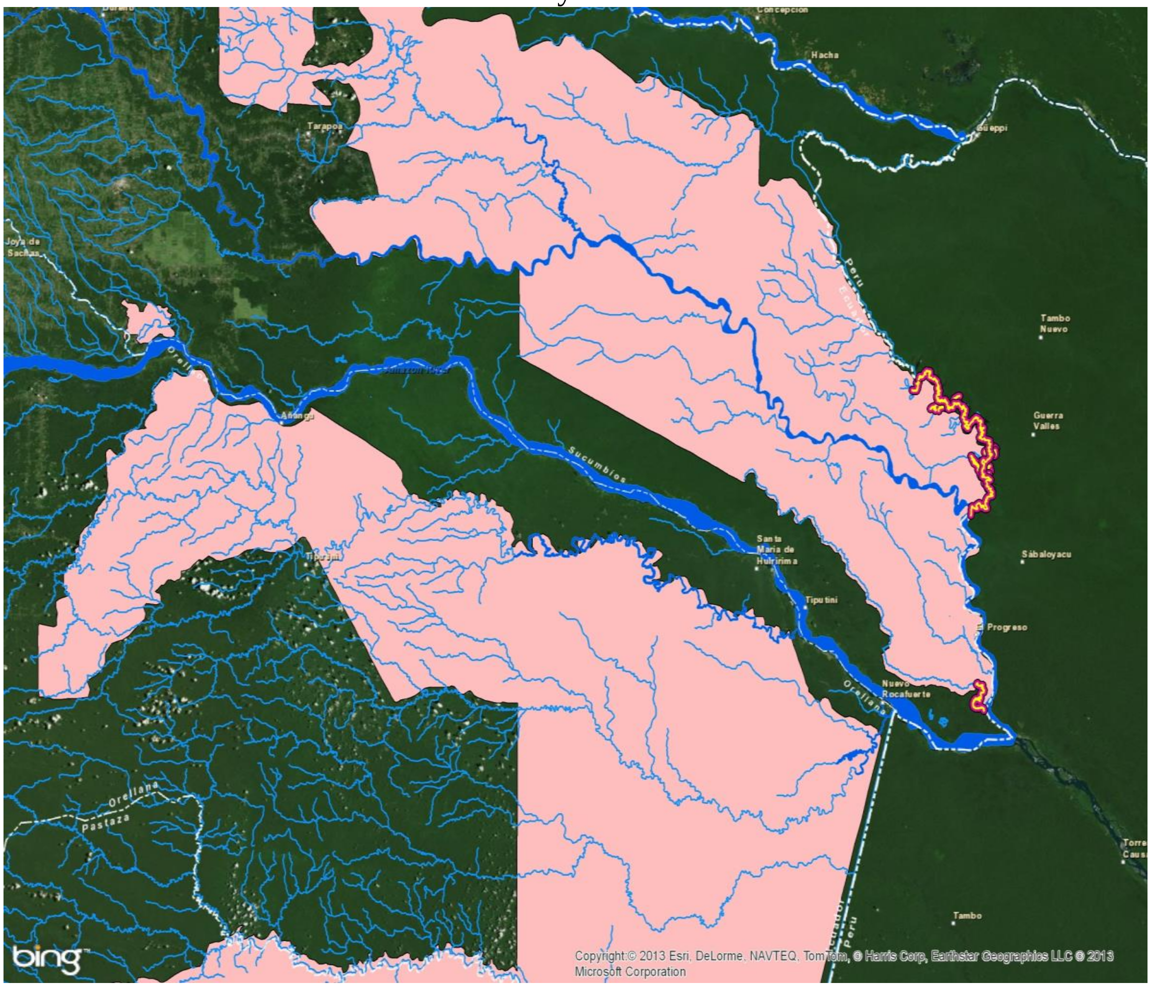
APPENDIX I

GARMIN® GPS SURVEY TRACKS

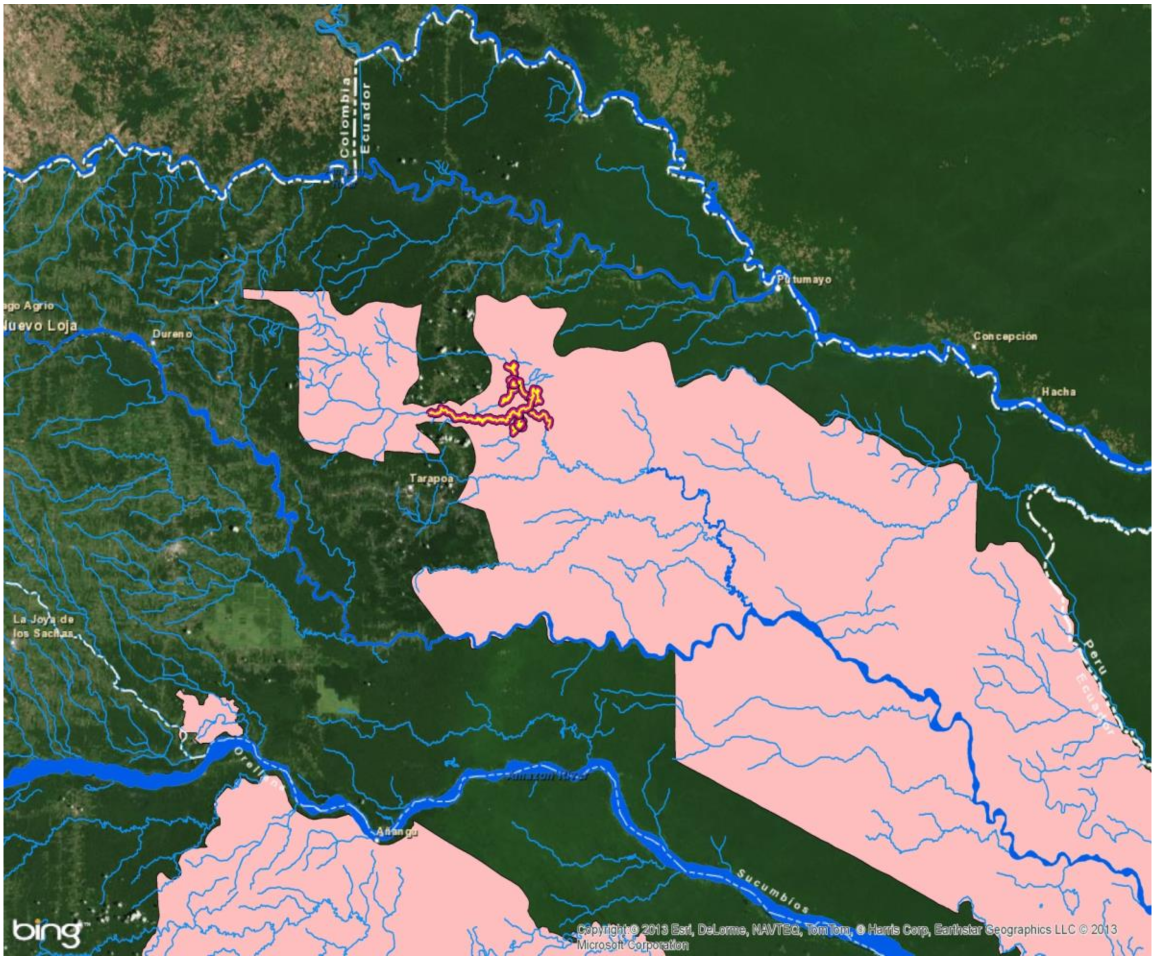
March 2011


Tarapoa
La Joya de los Sachas
San Sebastian del Coca
Puerto Francisco de Orellana
Añangu
Sucumbios
Santa Maria de Huiririma
Tiputini
Nuevo Rocafuerte
Orellana
Pastaza
Peru
Ecuador
Tambo
bing
Copyright © 2013 Esri, DeLorme, NAVTEQ, TomTom, © Harris Corp, Earthstar Geographics LLC © 2013 Microsoft Corporation

May 2011


Hacha
Tarapoa
Joya de Sachas
Tambo Nuevo
Guerra Valles
Sucumbios
Santa Maria de Huiririma
Tiputini
Sábaloyacu
Progreso
Nuevo Rocafuerte
Orellana
Pastaza
Tambo
Peru
bing
Copyright © 2013 Esri, DeLorme, NAVTEQ, TomTom, © Harris Corp, Earthstar Geographics LLC © 2013 Microsoft Corporation

July 2011


Colombia
Ecuador
Putumayo
Nuevo Loja
Dureno
Concepción
Hacha
Tarapoa
La Joya de los Sachas
Peru
Ecuador
Sucumbios
bing
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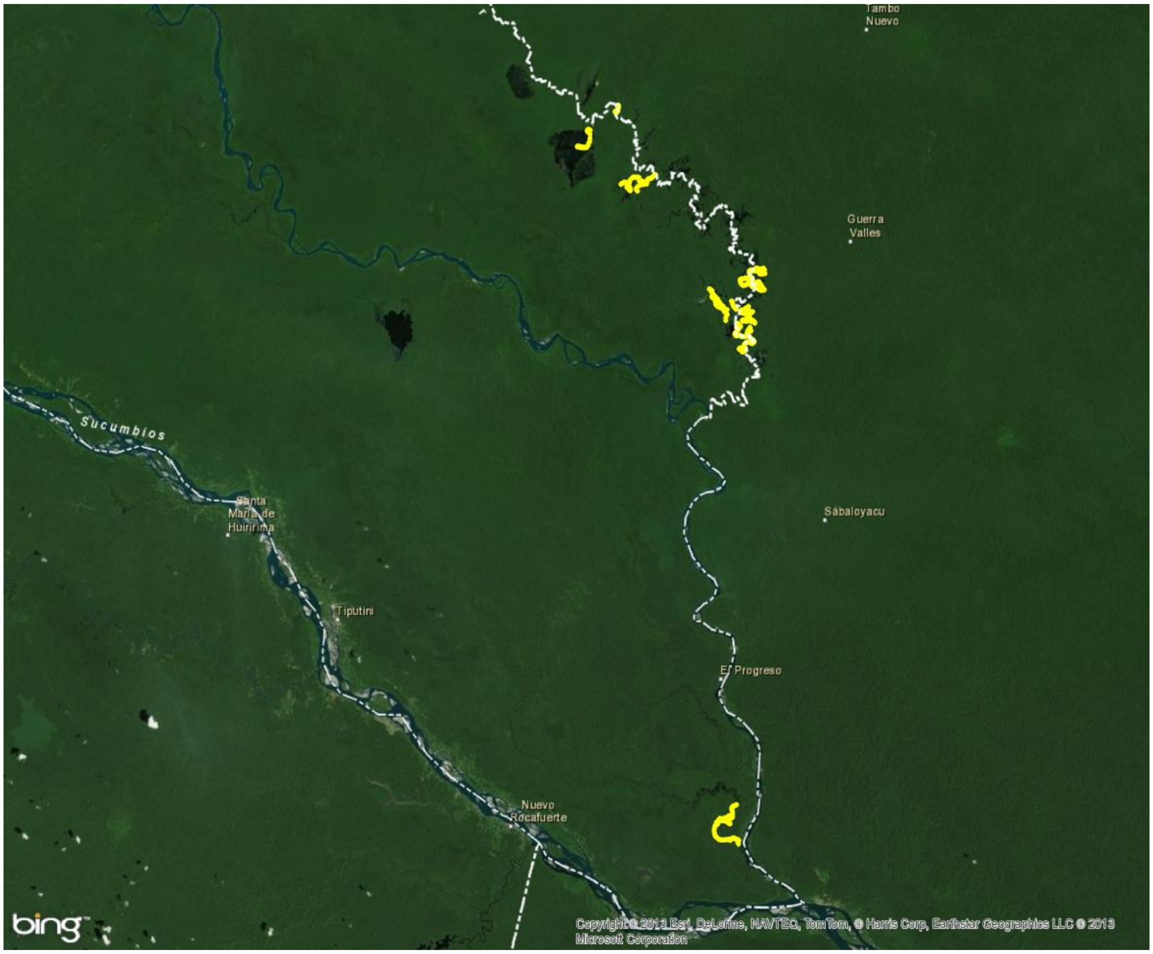
APPENDIX II

HUMMINBIRD® GPS SONAR RECORDING TRACKS

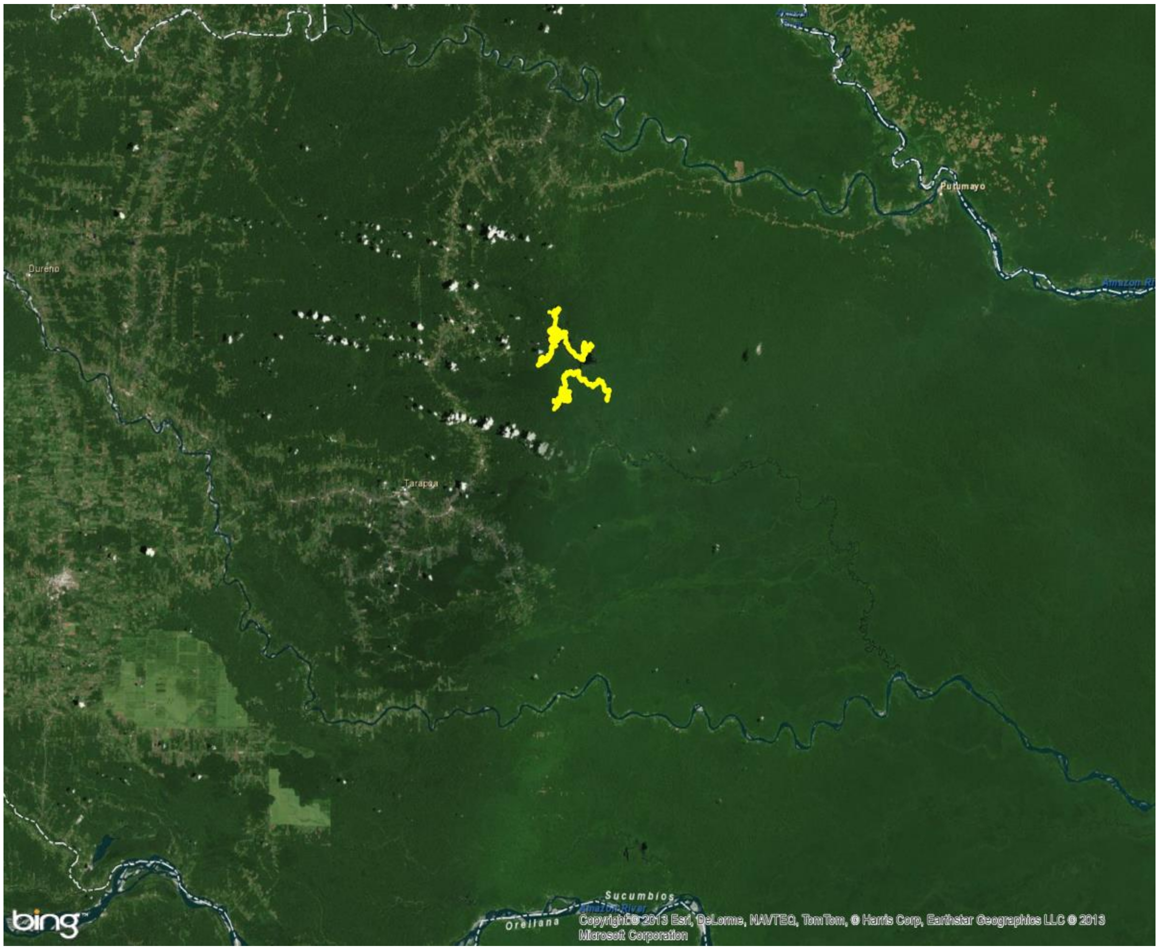
March 2011


Peru
Ecuador
Orellana
Amazon River
Añangu
Sucumbios
Santa
Maria de
Huiririma
Tiputini
Tiputini
Nuevo
Rocafuerte
Orellana
ellana
bing
Copyright © 2013 Esri, DeLorme, NAVTEQ, TomTom, © Harris Corp, Earthstar Geographics LLC © 2013 Microsoft Corporation

May 2011


Tambo Nuevo
Guerra Valles
Sucumbios
Santa Maria de Huiririma
Sábaloyacu
Tiputini
El Progreso
Nuevo Rocafuerte
bing
Copyright © 2013 Esri, DeLorme, NAVTEQ, TomTom, © Harris Corp, Earthstar Geographics LLC © 2013 Microsoft Corporation

July 2011


Putumayo
Dureno
Tarapoa
Sucumbios
Amazon River
Orellana
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APPENDIX III

HUMMINBIRD® SONAR RECORDINGS AND SCREEN CAPTURES

Memory Card Recording or Snapshot	Date/Time/Length (H:M:S)	Reviewed
Memory Card 1		
R00055	3/6/11 09:20 0:0:29	12/27/2012
R00056	3/6/11 10:42 0:3:57	12/27/2012
R00058	3/6/11 11:54 0:5:03	12/29/2012
R00060	3/6/11 12:21 0:3:28	12/29/2012
R00061	3/7/11 09:04 0:0:07	12/29/2012
R00062	3/7/11 09:04 01:06:29	12/29/2012
R00069	3/9/11 14:01 0:3:02	12/29/2012
R00070	3/10/11 09:00 0:0:32	12/29/2012
R00071	3/10/11 12:49 0:38:24	12/29/2012
S00002	Information on Instrument	12/29/2012
S00003	Information on Instrument	12/29/2012
S00004	Information on Instrument	12/29/2012
S00005	Information on Instrument	12/29/2012
S00006	Information on Instrument	12/29/2012
S00007	Information on Instrument	12/29/2012
S00008	Information on Instrument	12/29/2012
S00009	Information on Instrument	12/29/2012
S00010	Information on Instrument	12/29/2012
S00011	Information on Instrument	12/29/2012
S00012	Information on Instrument	12/29/2012
S00013	Information on Instrument	12/29/2012
S00014	Information on Instrument	12/29/2012
S00015	Information on Instrument	12/29/2012
S00016	Information on Instrument	12/29/2012
S00017	Information on Instrument	12/29/2012
S00018	Information on Instrument	12/29/2012
S00019	Information on Instrument	12/29/2012
S00020	Information on Instrument	12/29/2012
S00021	Information on Instrument	12/29/2012
S00022	Information on Instrument	12/29/2012
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S00024	Information on Instrument	12/29/2012
S00025	Information on Instrument	12/29/2012
S00026	Information on Instrument	12/29/2012
S00027	Information on Instrument	12/29/2012
S00028	Information on Instrument	12/29/2012
S00029	Information on Instrument	12/29/2012
Memory Card 6		

R00076	5/23/11 13:31 0:15:19	12/29/2012
R00077	5/23/11 13:52 0:0:39	12/29/2012
R00078	5/23/11 13:55 0:58:58	12/29/2012
R00080	5/23/11 16:26 0:15:18	1/5/2013
R00082	5/24/11 12:23 0:55:32	1/5/2013
R00083	5/24/11 13:18 0:21:46	1/5/2013
R00084	5/24/11 13:41 0:45:15	1/5/2013
R00085	5/24/11 14:31 0:8:32	1/5/2013
R00086	5/24/11 14:53 (Length Corrupt)	1/5/2013
R00087	5/24/11 14:53 0:16:01	1/5/2013
R00089	(Date/Time Corrupt) 0:27:01	1/5/2013
R00090	5/25/11 11:04 0:41:45	1/5/2013
R00091	5/25/11 11:46 0:39:08	1/5/2013
R00092	5/25/11 12:45 0:54:12	1/5/2013
R00093	5/25/11 13:51 0:52:16	1/5/2013
R00094	5/26/11 09:56 1:01:38	1/5/2013
R00095	5/26/11 11:08 0:16:51	1/5/2013
R00096	5/26/11 11:34 0:23:06	1/5/2013
R00097	5/26/11 12:57 0:11:49	1/5/2013
R00098	5/26/11 13:50 0:2:12	1/5/2013
R00099	5/26/11 13:55 0:20:43	1/5/2013
R00100	5/26/11 14:18 0:9:46	1/9/2013
R00101	5/26/11 14:32 0:55:02	1/9/2013
R00102	5/26/11 15:46 0:7:42	1/9/2013
R00103	5/26/11 16:08 0:7:00	1/9/2013
R00104	5/27/11 09:53 0:54:40	1/9/2013
R00105	5/27/11 10:50 0:27:43	1/12/2013
R00106	5/27/11 11:21 0:40:40	1/12/2013
R00107	5/27/11 12:13 0:23:35	1/12/2013
R00108	(Date/Time Corrupt) 0:24:14	1/12/2013
R00109	5/28/11 12:46 0:3:12	1/12/2013
R00110	5/28/11 12:50 0:57:42	1/13/2013
R00111	5/28/11 13:48 0:31:29	1/13/2013
S00031	Information on Instrument	1/13/2013
S00032	Information on Instrument	1/13/2013
S00033	Information on Instrument	1/13/2013
S00034	Information on Instrument	1/13/2013
S00035	Information on Instrument	1/13/2013
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S00037	Information on Instrument	1/13/2013
S00038	Information on Instrument	1/13/2013

S00039	Information on Instrument	1/13/2013
S00040	Information on Instrument	1/13/2013
S00041	Information on Instrument	1/13/2013
S00042	Information on Instrument	1/13/2013
S00043	Information on Instrument	1/13/2013
S00044	Information on Instrument	1/13/2013
S00045	Information on Instrument	1/13/2013
Memory Card 7		
R00115	(Date/Time Corrupt) 0:30:14	1/13/2013
R00116	(Date/Time Corrupt) 0:3:28	1/13/2013
R00118	(Date/Time corrupt) 0:7:29	1/13/2013
R00120	7/10/11 12:36 0:4:05	1/13/2013
R00121	7/10/11 12:55 0:27:24	1/13/2013
R00122	7/10/11 13:34 0:31:17	1/13/2013
R00123	7/11/11 09:51 0:28:28	1/15/2013
R00124	7/11/11 10:28 0:10:42	1/15/2013
R00125	7/11/11 10:39 0:21:47	1/15/2013
R00126	7/11/11 11:03 0:29:03	1/18/2013
R00127	7/11/11 11:33 0:32:48	1/18/2013
R00128	7/11/11 12:13 0:32:38	1/21/2013
R00129	7/11/11 12:52 0:25:31	1/21/2013
R00130	(Date/Time Corrupt) 0:13:24	1/21/2013
R00131	7/12/11 10:09 0:5:58	1/21/2013
R00132	7/12/2011 10:24 1:43:17	1/21/2013
R00134	(Date/Time Corrupt) 0:49:32	1/22/2013
Memory Card 8		
R00136	(Date/Time Corrupt) 1:21:55	1/23/2013
R00137	7/13/11 12:06 1:40:42	1/23/2013
R00140	(Date/Time Corrupt) 1:01:44	1/23/2013
R00142	(Date/Time Corrupt) 0:52:16	1/24/2013
Total recordings (with Usable Data): 63 Screen Captures: 43		

VITA

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Address: 4221 W. McNab Apt. 40, Pompano Beach, FL 33069

Email: cb1383@nova.edu

EDUCATION:

Master of Science in Marine Biology, Nova Southeastern University, 2014
GPA: 3.79
GRE: Verbal – 530, Quantitative – 750, Writing – 4.5

Bachelor of Arts in Biology and Chemistry (with Honors), Drury University, 2009
Minor in Global Studies, GPA 3.95, Summa Cum Laude
ACT: 31

PROFESSIONAL EXPERIENCE:

Florida Spectrum Environmental Services, Inc., Quality Assurance Director, Dec. 2012-Present

- Direct, enforce, and audit certified environmental laboratory's quality system, procedures, data, reports, and analyst competency for conformance to NELAC, FDOH, FDEP, and EPA requirements and client specific objectives.

Florida Spectrum Environmental Services, Inc., Analytical Chemist and Microbiologist, Feb. 2010 – Dec. 2012

- Performed EPA methods in inorganics, organics, extractions, and microbiology in solid, water, and chemical matrices.
- Analyzed samples using HPLC, IC, GC/MS, Flow Analyzers, Discrete Analyzers, Block Digesters, Distillation Units, and Incubators.

Bass Pro Shops Wonders of Wildlife Museum, Intern, May 2007-Aug. 2007

- Handled animal care, veterinary care, and water quality analysis of indoor aquaria and enclosures.

TEACHING EXPERIENCE:

Nova Southeastern University, Farquhar College of Arts and Sciences, Laboratory Assistant, Aug. 2009-May 2011

- Assisted with the set-up and teaching of Biology 101 laboratory classes.

RESEARCH EXPERIENCE:

Nova Southeastern University, Graduate Research Assistant, 2010-2011

- Collected water samples, conducted interviews, and performed side-scan sonar surveys to detect Amazonian Manatees and investigate their population ecology in the rivers and lagoons of the Amazon River basin in Eastern Ecuador.

Newfound Harbor Marine Institute Seacamp, Research Intern, May 2006

- Snorkeled and surveyed marine life in Big Pine Key, FL.

PRESENTATIONS:

Florida Marine Mammal Health Conference, Poster Presenter, April 2012, "Oil Effects on the Amazonian Manatee (*Trichechus inunguis)* in Eastern Ecuador: Evaluating the Risks"

Southeast and Mid Atlantic Marine Mammal Symposium, Oral Presenter, March 2012, "Oil Effects on the Amazonian Manatee (*Trichechus inunguis*) in Eastern Ecuador: Evaluating the Risks"

19th Biennial Conference on the Biology of Marine Mammals, Oral Presenter, Nov. 2011, "The Status of Amazonian Manatees (*Trichechus inunguis*) and Their Habitats in Eastern Ecuador"

Fifth Annual International Sirenian Symposium, Oral Presenter, Nov. 2011, "The Status of Amazonian Manatees (*Trichechus inunguis*) and Their Habitats in Eastern Ecuador"

PUBLICATIONS:

Brice, Caitlin. October 2011. "The Status of Amazonian Manatees (*Trichechus inunguis*) and Their Habitats in Eastern Ecuador". Sirenews. Number 56. www.sirenian.org/sirenews/56OCT2011.pdf

SKILLS:

Trained Protected Species Observer (PSO)
Certified PADI Open Water Diver
Proficient with Humviewer®, ArcGIS®, Agilent ChemStation®, and Distance® software
Intermediate Spanish Reading, Writing, and Conversational Skills

HONORS AND AWARDS:

First Place - Southeast and Mid Atlantic Marine Mammal Symposium (2012)
Walter H. Hoffman Chemistry Award for outstanding academic performance (2009)
Beta Beta Beta, Chi Chapter Inductee – Biology Honor Society (2008)
Omicron Delta Kappa – Leadership Honor Society Inductee (2008)
National Honor Society (Inducted 2003)
Concert Master – Drury Chamber Orchestra and Drury String Quartet (2007-2009)
Girl Scout Gold Award Recipient (2008)
Dean's List all four years of college (2005-2009)
Community Leadership Scholarship (2005)
Elk's Lodge Scholarship (2005)
Presidential Academic Scholarship (2005)
Presidential Musical Scholarship for Violin (2005)
Women in Science Award (May 2005)

ORGANIZATIONS AND AFFILIATIONS:

Marine Animal Rescue Society (MARS) – Trained Volunteer
Florida Society of Environmental Analysts (FSEA)
The NELAC Institute (TNI)
The Society of Marine Mammalogy
American Chemical Society