Evaluation of Ground Water from the Lajas Valley for Low Salinity Culture of the Pacific White Shrimp *Litopenaeus vannamei*

by

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Abstract

The ability of the Pacific white shrimp (*Litopenaeus vannamei*) to acclimate to and survive in low salinity ground water from southwestern Puerto Rico was evaluated using post larval shrimp and well water from the Lajas Valley. Five well sites were selected. Salinity ranged from 0.7-9.8 parts per thousand (ppt). Concentrations of (Na, Cl, K, Ca, Mg, SO_4, CO_3, and Mn) were determined for each water source. Comparing this with data from results reported in 1991 indicate the deep aquifer (> 45 m) is stable. Eighteen day old post larvae (PL_{18}) were used in 48 h bioassay acclimation experiments. Results were favorable in 2 of 5 well sites with survival ranging from > 99-0%. Survival was highest in low salinity waters (0.7-3.5 ppt). Potassium was deemed deficient in waters with low survival. Adjustment of the Na/K ratio was achieved by addition of potassium rich fertilizer. Two sites (salinities 6.7 and 9.8 ppt respectively) were tested with and without addition of salts (K_2SO_4 and KCl) to assess the role of Na to K ratios on 48 h survival of post larval shrimp. Survival was assessed in a second 48 h acclimation trial. The two well waters with nothing added served as controls. K levels raised to 3.6% of Na had > 99% survival and the controls had 0-18% survival over 48 h. Results were highly significant (ANOVA, $p < 0.001$) and were considered positive as the addition of K_2SO_4 and KCL ameliorated the mortality problems. Subsequently, a recirculating system (1154 L) was constructed to examine long-term survival. Water from well site # 2 (6.7 ppt) was used. The concentration of K to Na was raised to 3.6% by adding K_2SO_4 and shrimp were acclimated. Nursed juvenile shrimp (30 mm) were stocked into the system at a density of 0.32 shrimp / L. Shrimp lasted over one year in this system, and some grew to over 20 g indicating the feasibility of growing *L. vannamei* in these low salinity ground waters with proper compensation for low K content.

Resumen

Para evaluar la habilidad del camarón blanco *Litopenaeus vannamei* de aclimatarse y sobrevivir la bajo salinidad del agua de pozo, en el suroeste de Puerto Rico, se hicieron experimentos usando agua de pozo del Valle de Lajas. Seleccionamos 5 pozos. La salinidad de estos pozos fluctúa entre 0.7-9.8 partes por mil (ppt por sus siglas en ingles). Analizamos los minerales en el agua. Se determinó la concentración de los iones (Na, Cl, K, Ca, Mg, SO_4, CO_3, y Mn). Esto se comparó con data reportado en 1991 para concluir que los acuíferos profundos (> 45 m) están estable. Se usaron camaroncitos, cuya edad era 18 días después de ser post larva (PL_{18}) para un experimento de aclimatación de 48 horas. El resultado fue favorable en 2 de 5 pozos. La sobre vivencia del PL fue buena > 99% en las aguas con salinidad baja (0.7 y 3.5 ppt). Se determinó que el elemento potasio (K) hacia falta en las muestras con baja sobre vivencia. Para subir la cantidad de K en las muestras, echamos abono rico en K; usando el sulfato de potasio (K_2SO_4) o el cloro de potasio (KCl). Estos dos pozos (6.7 y 9.8 ppt) se usaron para otro experimento, pero ahora con y sin las sales ajustadas. El experimento duró 48 horas y se calculo la sobre vivencia. El control contenía agua natural del pozo. Los resultados indican que con la adición del abono la sobre vivencia subió a > 99% lo cual es mucho mejor que el control en que murieron casi todos. En general, los resultados se consideran muy positivos y eran muy significativos (ANOVA, $p < 0.001$). Luego, se construyó un sistema de recirculación de 1154 L para examinar la sobre vivencia a largo plazo. Este sistema se llenó con agua del pozo # 2 (6.7 ppt). La concentración de K a Na se subió a 3.6% añadiendo K_2SO_4 y los camarones fueron aclimatados. Los 300 camarones jóvenes (30 mm) fueron sembrados a una densidad de 0.32 camarones / L. Los camarones duraron poco más de un año en el sistema y algunos alcanzaron hasta 20 g de peso, indicando que es viable usar agua del pozo para cultivar *L. vannamei* siempre cuando se compensa para la deficiencia de K.

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For Lara Christina

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Table of Contents

ABSTRACT	……………………………………………………………………	ii
RESUMEN	……………………………………………………………………	iii
COPYRIGHT	……………………………………………………………………	iv
DEDICATION	……………………………………………………………………	v
ACKNOWLEDGEMENTS	……………………………………………………………	vi
TABLE OF CONTENTS	……………………………………………………………	vii
LIST OF ABBREVIATIONS	…………………………………………………….	viii
LIST OF TABLES	……………………………………………………………………	x
LIST OF FIGURES	……………………………………………………………………	xi

CHAPTER 1	BACKGROUND INFORMATION ………………………………	1
1.1	INTRODUCTION ………………………………………………	1
1.2	LITERATURE REVIEW……………………………………………..…	8
1.3	WATER RESOURCES IN THE LAJAS VALLEY …………………………	18
1.4	GEOLOGY OF THE STUDY AREA………………………………………	23
1.5	SUMMARY AND OBJECTIVES …………………………………………	32
CHAPTER 2	ACCLIMATION TO GROUND WATER ………………………...	34
2.1	INTRODUCTION PILOT STUDY ……………………………………….	34
2.2	MATERIALS AND METHODS - SITE SELECTION…..……………………	38
2.3	MATERIALS AND METHODS - ACCLIMATION STUDY ……………...	46
2.4	RESULTS ………………………………………………………	48
2.5	DISCUSSION ………………………………………………………	52
2.6	CONCLUSIONS ………………………………………………………	55
CHAPTER 3	ACCLIMATION TO GROUND WATER WITH ADDED POTASSIUM	58
3.1	BALANCING IONIC RATIOS ………………………………………	58
3.2	MATERIALS AND METHODS - K ION SUPPLEMENTATION ………..	60
3.3	RESULTS ………………………………………………………	62
3.4	DISCUSSION AND CONCLUSIONS ………………………………	64
CHAPTER 4	LONG TERM GROWTH AND SURVIVAL……………………………	66
4.1	INTRODUCTION ………………………………………………	66
4.2	MATERIALS AND METHODS ………………………………………	68
4.3	RESULTS ………………………………………………………	69
4.4	DISCUSSION ………………………………………………………	70
4.5	CONCLUSIONS ………………………………………………………	78

REFERENCES	……………………………………………………………………	81
APPENDIX	………………………………..………………………..	87

List of Abbreviations *

AkA	Aguirre Clay
ANOVA	Analysis of Variance
BMP	Best Management Practices
BOD	Biological Oxygen Demand
BV	Baculo Virus
Ca	Calcium
CAS	Control Aquaculture System
CeA	Cartagena Clay
Chl-a	Chlorophyll a
Cl	Chloride
CO_2	Carbon Dioxide
CO_3	Carbonate
DO	Dissolved Oxygen
FAO	Food and Agriculture Organization
FeA	Fe Clay
FrA	Fraternidad Clay
FWS	Free Water Surface
GnA	Guanica Clay
h	Hour
HACCP	Hazard Analysis Critical Control Point
ha	Hectare
IHHNV	Infectious Hypodermal and Hematopoietic Necrosis Virus
K	Potassium
kg/m^2	Kilogram per Meter Squared
KCl	Potassium Chloride
K_2SO_4	Potassium Sulfate
L. vannamei	*Litopenaeus vannamei*
LC50	Lethal Concentration 50%
lx	Lux
Mg	Magnesium
Mgal/d	Million Gallons per Day
mg/L	Milligrams per Liter
mmhos/cm	Milliohms per Centimeter
mmol	Milli Mole
Mn	Manganese
mOsm	Milli Osmole
mt	Metric Tons
Na	Sodium
NaCl	Sodium Chloride
NH_4	Ammonia
NHP	Necrotizing Hepatopancreatitis
NO_2	Nitrite
NO_3	Nitrate
PCR	Polymerase Chain Reaction

PL	…………………………………………………………………...	Post Larvae
ppt	…………………………………………..............................	Parts per Thousand
ppm	……………………………………….................................	Parts per Million
PVC	………………………………………………………………	Poly Vinyl Chloride
RAS	………………………………………………	Recirculating Aquaculture System
SF	…………………………………………………………………	Surface Flow
SO_4	……………………………………………………………………..	Sulfate
SOP	……………………………………………………….	Standard Operating Procedure
SPF	………………………………………………………….	Specific Pathogen Free
SS	………………………………………………………………	Suspended Solids
TAN	………………………………………………………..	Total Ammonia Nitrogen
TSV	………………………………………………………….	Taura Syndrome Virus
USFWS	………………………………………..	United States Fish and Wildlife Service
USGS	………………………………………………..	United States Geological Service
µS/cm	………………………………………………………….	Micro Siemens per cm
UV	……………………………………………………………………..	Ultra Violet
WSSV	…………………………………………………….	White Spot Syndrome Virus
YHV	…..	Yellow Head Virus

* Commonly understood abbreviations (cm, d, kg, mm, and y) are not included.

List of Tables

Tables **Page**

Table 2.1 Water quality data from 1991 and 2005 for selected wells in the Lajas Valley. 48

Table 2.2 Salinity vs. 48 h Survival. ……………………………………………... 49

Table 2.3 AOV table for all treatments and for each experimental treatment vs. control. 50

Table 3.1 Concentration of K in Relation to Na from Sites 2 and 4, and Amount of K_2SO_4 and KCl Added to 76 L.………………………………………… 61

Table 3.2 Survival in Treated vs. Untreated Well Water. …………………………… 62

Table 3.3 AOV table for treatments of added K vs. pure well water. ……………….. 63

List of Figures

Figures **Page**

Figure 1.1 Ground Water Levels and Precipitation for the Lajas Experimental Station. 20

Figure 1.2 Geology of the South Coast Ground Water Province. ……………...……… 24

Figure 1.3 Location of Five Study Sites in the Lajas Valley, With Close Up of Site # 1. 26

Figure 1.4 Soil Types Near Sample Site # 1. ……………………………………….. 27

Figure 1.5 Soil Types Near Sample Site # 2. ……………………………………….. 28

Figure 1.6 Soil Types Near Sample Site # 3. ……………………………………….. 29

Figure 1.7 Soil Types Near Sample Site # 4. ……………………………………….. 30

Figure 1.8 Soil Types Near Sample Site # 5. ……………………………………….. 31

Figure 2.1 Minimum ionic Concentrations Required for Low Salinity Shrimp Culture. 36

Figure 2.2 Google Earth Image Indicating Locations of Wells Sampled in Lajas Puerto Rico. ………………………………………………………….. 38

Figure 2.3 Well Site # 1 On the Cartagena USFWS Refuge. …………………………. 40

Figure 2.4 Well Site # 2 On the UPR-RUM Experimental Agriculture Station. ……… 41

Figure 2.5 Well Site # 3 On a Hay Farm Near Road 116, Bo. Sabana Yegua. ...……..... 42

Figure 2.6 Well Site # 4 On a Hay Farm south of Road 322 int. Bo. Lajas Arriba. …… 43

Figure 2.7 Well Site # 5 On Range Land South of Road 327 int. Bo. La Plata. ……… 44

Figure 2.8 Well Depths and Screened Sections of the Five Selected Wells. ………….. 45

Figure 2.9 Aquarium Rack showing Experimental Design. …………………………… 47

Figure 2.10 Salinity Measurements During Low Salinity Acclimation. …………..….... 49

Figure 2.11 Average Survival After 48 h Low Salinity Acclimation. ………………….. 51

Figure 2.12 Average Number of Live Shrimp During the 48 h Low Salinity Acclimation. 51

Figure 2.13 Soil Types Near a Proposed Future Study Site. ………………………..…… 56

Figure 3.1 Average 48 h Survival in Water With and Without Added K. …………...… 62

Figure 3.2 Average Survival During Acclimation to Water With and Without Added K. 63

Figure 4.1 Schematic Diagram of the Recirculating System Used for Grow Out Study. 68

Figure 4.2 Survival and Growth Over 1.25 Y in a Recirculating System. ...………….... 69

Figure 4.3 Model of a Low Salinity Shrimp Culture Demonstration Facility. ……..….. 79

Figure A.1 Configuration of Bedrock Surface Underlying Alluvial Sediments in Lajas. 87

Figure A.2 Wells Surveyed in the Lajas Valley between 1983-1986. …………………. 88

Figure A.3 Map of the Lajas Valley Agricultural Reserve. ……………………………. 88

CHAPTER 1 – BACKGROUND INFORMATION

1.1 Introduction

Many marine fisheries world-wide have been depleted to the point of economic extinction (Platt 1994), and according to Matos (1997) the artisanal fishery in Puerto Rico appears to be suffering a similar fate. Matos (1997) reported fish and shellfish production for 1994 to 1996 was 1.2-1.7 million kg, which is a drastic drop from the 1979 to 1982 average of 2.9 million kg (Collazo and Calderon 1988). However, worldwide aquaculture has been the fastest growing food production system, with an average compound growth rate of 9.6% since 1986 compared to the 3.1% annual growth rate for terrestrial livestock meat and 1.6% for capture fisheries (Dudley-Cash 1998). FAO (2005) reports that aquaculture is an important food-producing sector. It provides much needed protein, employment, income and livelihoods that support many people in the world. Shrimp, in particular, is a high value commodity that is mainly produced in Asia and Latin America, especially for export, and brings a wealth of revenue to many developing countries in those regions.

Litopenaeus vannamei has been widely cultured commercially around the world as a food product (Teichert-Coddington 2002). FAO (2007) statistics show that total worldwide farmed production of *L. vannamei* increased steadily from 8000 mt in 1980 to 194 000 mt in 1998. After a small decline in 1999 and a more significant decline in 2000 due to the arrival of WSSV in Latin America, a rapid increase in shrimp production occurred to over 1 386 000 mt in 2004. The top four producer countries in 2004 were: China (700 000 mt), Thailand (400 000 mt), Indonesia (300 000 mt) and Vietnam (50 000 mt) (FAO 2007). Total production of this species in all of the Americas amounted to some 213 800 mt, worth US $1.1 billion in 2002 (Briggs et al., 2004). Unfortunately, this growth in shrimp production in many cases has been uncontrolled and unregulated. As a result of this, some serious problems have arisen.

Historically, marine shrimp farms were located exclusively in coastal areas due to the requirement for salt water. Coastal development of marine shrimp operations caused several significant problems. One of these problems was the destruction of mangroves. Mangroves exist along tropical and subtropical sheltered coasts, which is also where most shrimp farms are constructed. Mangroves are classified as diverse ecosystems and are important to both humans and animals. According to Carvajal and Alava (2007), mangroves are one of the most important and productive coastal wetlands in the world which have multiple benefits including: protection against hurricanes, storms and flooding; are natural water treatment systems and sedimentation sinks, in addition to supporting local economies with eco-tourism and fisheries. In Ecuador, mangroves provide nursery areas and habitats for several species of crustaceans, including the Pacific white shrimp, *Litopenaeus vannamei*. Other reported species that inhabit coastal mangroves in Ecuador are: Pacific blue shrimp *Litopenaeus stylirostris*; red crab, *Ucides occidentalis*; fishes, including croakers, *Cynoscion* spp.; mullets, *Mugil* spp. (e.g., *Mugil cephalus*), and snook, *Centropomus nigrescens*; shellfishes, including mussels, *Mytella strigata* and *M. speciosa*; and mangrove cockles (ark shells), *Anadara* spp. (e.g. *Anadara tuberculosa*, *A. similis* and *A. grandis*); reptiles, such as the critically endangered American crocodile, *Crocodylus acutus* and green iguanas, *Iguana iguana*; birds, including mangrove black hawk, *Buteogallus anihmcinus*; white ibis, *Eudocimus albus*; roseate spoonbill, *Ajaia ajaja*; and herons, including great egrets, *Ardea alba*; snowy egrets *E. thula*; little blue herons, *E. caerulea*; tricolored herons, *E. tricolor*, green-backed herons, *Butorides striatus*, as well as black-crowned *Nycticorax nycticorax* and yellow-crowned *Nyctanassa violacea* night herons; and, mammals, including crab-eating raccoon, *Procyon cancrivorus*, and neotropical otter, *Lontra longicaudis*. Bottle-nose dolphins (*Tursiops truncatus*) are also common marine mammals residing around the mangrove estuarine waters along the Ecuadorian coast. Of the six species of mangrove reported in Ecuador, all species are threatened and four of these species (red mangroves, *Rhizophora mangle* and *R. harrisonii*; black mangrove, *Avicennia germinans*; white mangrove, *Laguncularia racemosa*; button or jeli mangrove, *Conocarpus erectus*) are currently at risk of extirpation (Carvajal and Alava 2007). Moreover, mangrove forests, which once covered more than 200 000 km^2 of coastline, have been diminished by 35-86% in extent in locations around the world, according to FAO, and are critically endangered or approaching extirpation in 26 out of the 120 countries in which they are found (Wilkie and Fortuna 2003). Considerable areas of mangroves have been destroyed during development of shrimp ponds in India, Thailand, Costa Rica, and Ecuador. According to Menasveta (1997), in Thailand, about 54.7% of mangrove area was destroyed between 1961 and 1993. According to the most comprehensive survey to date (Wilkie and Fortuna 2003) on the state of the world's mangrove forests, the current mangrove area worldwide has now fallen below 15 million ha, down from 19.8 million ha in 1980. It also indicates that mangrove deforestation continues, albeit on a slightly lower rate in the 1990's (1.1%/y) than in the 1980's (1.9%/y), because most countries have now banned the conversion of mangroves for aquaculture purposes and require environmental impact assessments prior to large-scale conversion of mangroves for other uses (Wilkie and Fortuna 2003). Since mangroves are coastal ecosystems, moving shrimp farms inland away from coastal areas would benefit them directly.

Another serious problem that commercial shrimp farms have experienced to their great demise is viral pathogens such as: WSSV, IHHNV, TSV, BV, NHP, and YHV. Since the

1980's, viruses have been threatening the shrimp culture industry by devastating culture facilities worldwide (Liu et al., 2001). With this in mind, strict control of pathogenic material, especially untreated seawater, must be attained. Contaminated seawater is often the cause of disease outbreaks in previously unaffected areas. According to Flegel (2006), Asia leads the world in cultivated shrimp production with export earnings in the order of billions of US dollars per year. Despite this success, annual production decreased in the latter nineties because of widespread epizootics caused by viral pathogens. They reported that in Thailand, shrimp production trends have mirrored those in the rest of Asia, except that recovery from the viral epizootics has been somewhat better than it has been for most of its close neighbors. Since 2004, *L. vannamei* has been the dominant cultivated species in the world. Research in Thailand has focused on the characterization of shrimp viruses and on the development of rapid diagnostic probes for them. The major viruses of concern (in estimated order of past economic impact for Thailand) are WSSV, YHV, HPV, BV, TSV, and IHHNV (Flegel 2006). According to this research, the most rapid and sensitive tests employ PCR technology and take approximately 3 h to complete. However, lateral flow chromatographic tests based on nanogold-labeled monoclonal antibodies have recently been introduced. Although they tend to be less sensitive than PCR-based methods, they are highly specific, inexpensive and so user-friendly that they can be used pond-side by farmers themselves to verify disease outbreaks. However, even with the availability of affordable test kits and diagnostic procedures, it would be clearly beneficial to prevent the introduction of dangerous pathogens. Therefore moving shrimp culture facilities inland utilizing uncontaminated well water would be a clear improvement.

Finally, ocean front property is often prime real estate and therefore more expensive than comparable inland agricultural land. Coastal areas are also subjected to strong storms and flooding which can impact culture operations. Therefore, due to the problems associated with coastal culture of this marine species, it is apparent that inland aquaculture is more advantageous than traditional coastal culture techniques.

Fortunately, new discoveries have lead to alternative culturing techniques. One of the alternatives has been the use of saline ground water. Since saline well water occurs inland in some areas, using this resource frees the farmer from a mandatory locale next to the ocean. The culture of these shrimp in un-contaminated inland areas with virus free ground water has lead to excellent results (Liu et al., 2001). Bio-security has been coined as the phrase used to describe this type of control. This new culturing technique is a sustainable economic practice when strict bio-security protocols and BMP's are implemented (Samocha et al., 2001). According to FAO (2005), during the efforts to find lasting solutions to the disease problems affecting *L. vannamei* culture in Latin America, it was perceived that stocking with healthy post larvae is a key factor for achieving better survival during production. This requires a clear understanding of the basic principles of sound health management and hatchery bio-security. SOP and HACCP type interventions can be applied during hatchery production of *L. vannamei* post larvae. This is expected to facilitate the efforts of hatchery operators and managers to produce quality, disease-free, healthy *L. vannamei* post larvae, thus improving overall production and the sustainability of white shrimp aquaculture.

Since ground water is free from these dangerous pathogens, it is a desirable resource to use. When SPF shrimp are exclusively imported to inland, virus free facilities, bio-security can be obtained. According to Scura (2007), SPF shrimp are produced in viral-free hatcheries and certified free from the following category 1 pathogens: WSSV, IHHNV, TSV, BV, NHP, and YHV. In some cases, other pathogens may be included to this list, depending on the source of the SPF shrimp. Currently, Puerto Rico has no operating marine shrimp farms. This situation further reduces the probability of contamination of pathogenic viruses to an inland culture facility since the presence of neighboring shrimp farms increases the chance for viral contamination via avian vectors. Historically, Eureka Marine Inc. (a 300 acre shrimp farm) operated in Dorado, PR with good production success. They never reported problems with viral pathogens. However, during 2004-2005 this company began to import SPF post larvae from Florida as a preventative measure.

Unfortunately, inland culture of *L. vannamei* using low salinity well water has not been completely successful. Several problems arose when aquaculturists began raising these marine species in low salinity ground waters. The most pronounced problem faced was the variable ionic concentrations (and salinities) in ground water. Ground water is or becomes saline for 7 principal reasons and can exhibit highly variable water quality. According to Richter and Kreitler (1993) on a regional level, the most important sources of saline ground water are: naturally occurring saline ground water, halite solution, seawater intrusion, oil field and gas brines, agricultural byproducts and techniques, saline seep, and road salt. For this research, naturally occurring saline ground water is the most important as this is the case in the Lajas Valley. Richter and Kreitler (1993) describe the origin of naturally occurring saline ground water as: residual (connate) water from the time of deposition in a saline environment, a solution of mineral matter in the unsaturated and saturated zones, concentration by evapotranspiration, intrusion of seawater, or any mixture of these. According to Graves (1991), the saline aquifer below the Lajas Valley (southwestern Puerto Rico) is residual (connate) water from the time of deposition. Historically, the Lajas Valley was connected to the Caribbean Sea through the Guanica Bay area. More information about this saline aquifer is discussed later in this chapter.

To summarize, saline aquifers can be highly variably depending on their origin and other man induced changes. Therefore, before any use is planned for a potential ground water resource, test wells must be constructed and water quality determined.

1.2 Literature Review

Aquaculturists have known for many years that *L. vannamei* can tolerate low salinity. Research of this nature was begun in Texas during 1972 when two gravel pit operators expressed interest in using saline ground water from some of their pits for aquaculture use. It started when Texas A&M University, County Extension Agent Johnny Harris stocked the first shrimp using low salinity inland water in 1973 demonstrating the biological feasibility of inland shrimp cultivation in west Texas (Treece 2002).

Worldwide Examples of Inland Low Salinity Marine Shrimp Culture

Since around 1998, aquaculturists have continued to research the potential of *L. vannamei* for culture in waters with salinities from < 1-10 ppt. Mendes and Pedreschi (1998) showed that in Brazil, these shrimp could be acclimated to fresh water with salinity as low as 0.09 ppt. In 1999, Harbor Branch Oceanic (Florida) offered classes on the low salinity culture of this species at their facility and even produced a detailed manual about the subject (Van Wyk et al., 1999). Other researchers have also reported that this species can be cultured almost entirely (except for the larvae stage) in waters with salinities as low as 2 ppt. According to Laramore et al. (2001) saline ground water and diluted seawater (2-30 ppt) can be substituted for full strength seawater (35 ppt). Panama has been experimenting with inland low salinity (0.8-2.5 ppt) culture techniques as a way of improving production in the presence of WSSV. Researchers harvested two ponds after 91 days post stocking, had mean shrimp weights of 13.49 and 15.09 g, total harvest weights of 1995 and 2917 kg/ha, and survival rates of 49.9 and 77.0% which was considered excellent and resulted in further stocking of 22 additional ponds (Pérez-Athanasiadis and García-Chávez 2002). According to Boyd et al. (2002) in Thailand, low salinity inland culture accounts for about 30% of the national production of *L. vannamei*. He states that due to this success, low salinity culture is being attempted in other countries including Ecuador, Brazil, and the United States. Since 2000, Ecuador has experienced a tremendous increase in inland shrimp farming. One report indicated that 60 inland farms had developed by 2002. Pond salinities at the most successful farm average 0.9-1.2 ppt, productions ranged from 4989-5896 kg/ha, and survivals ranged from 24-60% (Salame and Salame 2002). The United States has also been active in low salinity research with *L. vannamei*. In 2001, shrimp farmers in Texas produced 3500 mt of shrimp worth about $19.8 million to which inland production was a significant part. Production averages from inland farms in west Texas have ranged from 3.36-5.04 mt/ha, and during that time there were six inland farms operating with a total of 75 ha of water (Treece 2002). OceanBoy Farms in Florida is reported to be the largest and most successful marine shrimp farm that uses artisan ground water in the United States with a planned stocking of 70 million PL for the fall 2002 crop (McMahon et al., 2002). Samocha et al. (2002) reported excellent results (100% survival and good yield 2.3 kg/m^2) at a farm in Arizona, and also report that other farms in Israel are successfully using saline ground water with salinities ranging 1.8-2.6 ppt.

Improving Survival - PL Age and Ionic Ratios

Reports from the literature about low salinity shrimp culture using saline ground water are not all positive as in some cases, mortality was unexplainably high (Boyd et al., 2002). It has been reported that shrimp survival is drastically reduced when salinity is reduced below 0.18 ppt (Galli and Stern 1998). Laramore et al. (2001) reported that low survival observed at salinities below 2 ppt was an associated risk in culturing these marine shrimp at very low salinities but the reasons were not clear. Some of the survival variability shown in the literature may be related to the age of post larvae (PL) used in the experiments. McGraw et al. (2002) report a clear effect of

PL age on salinity tolerance. They showed that ten day old PL (PL_{10}) or younger could not successfully acclimate to less than 4 ppt, while PL_{15} and older PL can be acclimated to as low as 1 ppt. In addition to PL age, some of the reported variable survival may be due to variable ion concentrations found in inland ground waters. Seawater is not just NaCl and water. It is known that seawater contains additional ions such as Mg, Ca, K, SO_4, and CO_3 (Libes 1992). Whetstone et al. (2002) recommends at least 100 mg/L of potassium for the culture of this species. Davis et al. (2002) also reported that survival is affected by an interaction between PL age and concentrations of the major ions: Na, Cl, K, Mg, and SO_4. It has been shown that *L. vannamei* can not be cultured in water with the single salt NaCl even if it has total salinity of 35 ppt (Davis et al., 2004). Boyd (2003) reported that because Ca, Mg, and K have specific physiological functions, they are more important than Na, Cl, and SO_4 as these ions only contribute to salinity and osmotic pressure. In addition, Davis et al. (2004) report that although Na and Cl are the major ions used in osmoregulation, other ions such as K, Mg, and Ca play important roles in biochemical activities of the organism. They further explain that the ionic ratios of the major ions found in seawater are important. They report that as a general rule: salinity must be > 0.5 ppt, levels of Na, Cl, and K, are similar to what they would be for seawater diluted to that salinity, Ca is at least equal to K, and alkalinity is > 75 mg/L. Boyd et al. (2006) report that Alabama has low salinity wells that yield water deficient in potassium. They conclude survival can be improved by the addition of aqueous K, and Mg fertilizers, but discharge of these saline waters can cause problems in the environment.

Zero Discharge, Low Salinity Culture Options

In order to comply with environmental regulations restricting the discharge of saline waters, some farms recycle their water and have incorporated the reconditioning of effluents by storing discharge water in settling ponds containing several species of emergent plants to remove suspended solids; followed by two or three additional settling ponds stocked with water hyacinths (*Eichhornia crassipes*) to remove remaining nutrients (Salame and Salame 2002). OceanBoy Farms in Florida have Red Mangroves growing in their treatment ponds (McMahon et al., 2002). Montoya et al. (2002) report data from a pilot-scale constructed wetland unit, consisting of FWS and SF constructed wetlands arranged in series, which was integrated into an outdoor RAS for culturing Pacific white shrimp (*L. vannamei*). They evaluated the performance of the wetland unit in treating the recirculating wastewater and examined the effect of improvement in water quality of the culture tank on the growth and survival of shrimp post larvae. Data collected during an 80-d culture period showed the wetland unit effectively reduced the influent concentrations of 5-day BOD by 24%, SS were reduced by 71%, Chl-a was reduced by 88%, TAN fell 57%, NO_2 nitrogen decreased by 90%, and NO_3 nitrogen dropped 68%. The concentrations of SS, Chl-a, and NO_3 in the culture tank water in RAS were significantly ($P \leq 0.05$) lower than those in a CAS that simulated static pond culture without wetland treatment. In addition, harvest results showed that shrimp weight and survival rate in the RAS (3.8 +/- 1.8 g/shrimp and 90%) significantly exceeded ($P \leq 0.01$) those in the CAS (2.3 +/- 1.5 g/shrimp and 71%). Montoya et al. (2002) concluded that settling ponds constructed like wetlands can improve water quality and provide a good culture environment, consequently increasing shrimp growth and survival without water exchange, in a recirculating system. In addition, Gomez-Jimenez et al. (2005) evaluated the effect of dietary protein level on growth, survival and ammonia efflux rate of *L. vannamei* raised in a zero water exchange culture system. *L. vannamei* post larvae (1.96 +/- 0.07 g) were reared in a zero water exchange system for 25 days at 28^{o}C. They were fed four commercial diets containing 25, 30, 35 or 40% crude protein in three replicate aquaria per dietary treatment. Total ammonia, nitrite, nitrate and pH were monitored weekly and total ammonia levels were additionally measured every 3 d using the flow injection analysis method. Total ammonia efflux rates were measured at days 0, 14 and 21, and survival and growth rates were recorded at the end of the experiment. No significant differences between water quality parameters such as temperature, salinity, DO, and pH were found. Nitrite concentration remained low in all dietary treatments up to the second week increasing considerably from day 14 onwards suggesting the initiation of the nitrification process. Total ammonia of all experimental groups exhibited a gradual increase up to day 13; however, following this time ammonia levels of all experimental groups decreased, probably due to either the action of bacterial nitrification or ammonia-N uptake by the animals. High ammonia efflux rates were recorded at day 14, especially after the first hour of immersion in the 25% protein group, but no significant changes occurred in any experimental group after 3 h. No significant differences in weight gain, final weight or survival of shrimp were observed under these experimental conditions. Gomez-Jimenez et al. (2005) concluded that based on their data, water quality did not adversely affect shrimp performance in their zero exchange system. In addition, Cohen et al. (2005) discussed the characterization of water quality factors during intensive raceway production of juvenile *L. vannamei* using limited discharge and biosecure management tools. They explain that disease epizootics have negatively affected production and expansion of the shrimp culture industry. This, along with environmental concerns regarding limited water resources and contamination of receiving streams, has caused the industry to investigate more sustainable and biosecure management practices. Their study (Cohen et al., 2005) was conducted to evaluate the effect of limited water exchange on water quality, growth and survival of *L. vannamei* post larvae (PL) in greenhouse-enclosed raceways. Concentrations of NH_4 nitrogen did not exceed 2.0 mg/L during this period; whereas, NO_2 nitrogen exceeded 26.4 mg/L, indicating assimilation of primary amines by primary productivity. Periodic removal of SS by a common pressurized sand filter and injection of oxygen into culture water resulted in high estimated survival rates for both raceways (97.5 and 106.0%) with an average biomass yield of 4.29 +/- 0.06 kg/m^3. Shrimp samples collected during the nursery trial and at harvest showed no signs of bacterial or viral pathogen infections.

Optimal Conditions

Low salinity has actually been shown to be beneficial to this species. During specific life stages, *L. vannamei* naturally lives in estuaries (Gunter et al., 1964), consequently it has a wide tolerance for salinity fluctuations. It is generally believed that some marine species seek low salinity habitats such as estuaries when in the juvenile stage to help reduce the stress of high salinity induced osmotic pressure difference between their body fluid composition and full strength seawater. It has been reported that, in different areas of the world, waters with variable salinity (1-40 ppt) are being used to produce this species of marine shrimp (Davis et al., 2004). It has also been reported, that shrimps near the isosmotic point expend the least energy on respiration, osmoregulation, and excretion. Consequently they exhibit the best growth rate and the most efficient energy conversion at this salinity (Panikkar 1968). The isosmotic point for penaeids ranges from 23 to 30 ppt (Dall 1981, Castille and Lawrence 1981). Other studies have shown that holding penaeid shrimps at salinities near isosmotic points could result in an increase of food consumption (Staples and Heales 1991). However, the ideal salinity for culturing *L.*

Vannamei was not known until recently. Rosas et al. (2001) concluded that juveniles can tolerate changes in salinity and at lower salinities (15 ppt), the shrimp consumed 1.69 times more food than those at 5 ppt without an increase in respiratory metabolism which was tested by measuring the apparent heat increment. According to Wang et al. (2004), food consumption of *L. vannamei* generally increased with increasing salinity within the range of 1-16 ppt with the maximum value at 16 ppt, and was also the salinity at which the highest specific growth rate was observed. Gong et al. (2004) showed that the iso-osmotic point of shrimp was estimated to be 695.5 mOsm/kg, which was equivalent to 26.1 ppt in Arizona Mariculture Associates well water. Their higher salinity value presumably is due to ionic differences in the well water compared to standard seawater at that salinity. Zhang et al. (2006) reported on tolerance to hypoxic (low DO) of *L. vannamei* exposed to different temperatures (14.5, 21.5, 24.8, 27.8, 30.8, and 35.0^{o}C, salinities (9, 15, 26, 35, and 40 ppt), pH (3.3, 6.5, 7.7, 8.1, and 9.2), and light intensities (strong 2100 lx and weak 60 lx) at various body weights (3.0, 3.7, 4.3, 5.7, 7.8, 9.0, 9.5, 10.7, 11.9, and 13.3 g) and feeding conditions (fed for 3 h, fasted for 12 h, and fasted for 48 h) was measured. The following factors: body weight, temperature, salinity, and pH, had significant effects on lethal DO levels ($P < 0.01$). Not surprisingly, these results indicated that *L. vannamei* cultured at optimum conditions has the best ability to withstand hypoxia. Therefore, when possible, they suggested that water temperature be maintained at 22^{o}C, salinity 16.6 ppt, pH 7.56, and strong light conditions to help reduce mortality during low DO periods. Recently, Li et al. (2007) continued research using varied salinities, looking for the ideal salinity to culture these animals. They studied growth performance, body composition, respiration and NH_4 nitrogen tolerance of juvenile white shrimp, *L. vannamei*, at 3.0, 17.0 and 32.0 ppt. They found that after 50 days, weight gain at 17.0 ppt was the highest, and significantly higher ($p < 0.05$) than that of shrimps at 3.0 ppt. Shrimp survival rate at 3.0 ppt was significantly lower ($p < 0.05$) than that of other two groups. Hepatosomatic index and condition factor were not significantly ($p > 0.05$) affected by the ambient salinity. Shrimp body protein and ash content were not affected significantly ($p > 0.05$) by salinity, while body moisture increased at high salinity, and crude lipid in shrimps was lowest at 32.0 ppt. After being exposed to the above three salinities for 30 d prior to the test, shrimp oxygen consumption and respiratory quotient of the shrimps at 3.0 ppt were significantly higher ($p < 0.05$) than those of shrimps at medium and high salinities, while salinity did not significantly ($p > 0.05$) affect CO_2 production. When juvenile *L. vannamei* were exposed to seven NH_4 nitrogen concentrations (0, 4.00, 6.67, 9.33, 12.00, 14.67, and 17.33 mg/L) at the three above salinities to which shrimps had been separately acclimated for 10 d at pH 8.30 and 29^{o}C (+/- 0.5), shrimps at 3 ppt were the most susceptible to ambient NH_4 nitrogen, and the 96 h LC50 (95% confidence limit) to ambient NH_4 nitrogen was 9.33 (8.39-10.37) mg/L. Based on these results, Li et al. (2007) concluded that *L. vannamei* could adapt to a wide range of salinity, but the animals would be more susceptible to NH_4 toxicity and spend more energy to compensate the cost for osmoregulation at low salinity.

Physiology - Osmoregulation

Most marine invertebrates are generally considered osmoconformers. However, it has been shown that *L. vannamei* is an osmoregulator. The following reports explain to some extent the physiology of these shrimp related to low salinity acclimation. Sowers et al. (2006a) report that interest in culturing *L. vannamei* in low-salinity and brackish-well waters has led to questions about the ability of this species to osmo- and ionoregulate in environments containing low concentrations of ions and in environments with ionic ratios that differ from those found in sea water. After seven days, hemolymph osmolality and K, Na, and Ca values were all significantly affected by salinity (as artificial sea salt) with values decreasing with decreasing salinity. These decreases were small, however, relative to decreases in salinity, indicating ionoregulation and osmoregulation with adjustment for gradients. The hemolymph osmolality and Na and Ca concentrations in shrimp exposed to either 2 ppt artificial sea salt or 2 ppt mixed-ion solution (a mixture of Na, K, Ca, and Mg chlorides that approximate the concentrations and ratios of these cations found in 2 ppt dilute seawater) did not differ significantly. However, hemolymph K levels were significantly lower in shrimp held in the mixed-ion environment. K influx rates were similar in shrimp held in either artificial sea salt or mixed ions. The results of this study indicate that salinity affects hemolymph-cation concentrations and osmolality. Sowers et al. (2006a) concluded that differential K influx rates do not appear to be the basis for low hemolymph K levels observed in shrimp held in mixed-ion environments. Cheng et al. (2006) performed an 8-week feeding experiment to study the body composition of *L. vannamei* reared in seawater (28 ppt) and brackish water (2 ppt) respectively, including protein, lipid, ash, amino acid, fatty acid and mineral contents. No significant differences in protein and lipid contents of the whole body were observed among the shrimp reared in the two media, while the whole-body ash content of shrimp reared in brackish water was significantly lower than that in seawater. Judged from the mineral contents of exoskeleton, hepatopancreas, muscle and serum of shrimp reared in such salinity water, low salinity caused an inhibitory effect on the extent of tissue mineralization. The protein content in serum of shrimp reared in brackish water decreased significantly, while the hepatosomatic index increased. There were no significant differences in the total contents of amino acids and fatty acids in the muscle; however, the total content of essential amino acids increased significantly and the total content of unsaturated fatty acids decreased in the muscle of shrimp reared in brackish water. All of these suggested that the effect of salinity on body composition was a reflection of shrimp adaptation to saline media by osmotic and ionic regulation.

Conclusion

Therefore from this and other data, it has been concluded that *L. vannamei* is a strong osmoregulator effectively able to handle fluctuating salinities between 1-40 ppt, with the ideal salinity about 15-16 ppt. Lower salinities (< 15 ppt) are more easily adapted to when ionic ratios of the major ions present in seawater (Na, Cl, K, Mg, and Ca) are similar to standard seawater as reported by (Libes 1992). Consequently, over the last few years, marine shrimp mariculture has been shifting away from coastal areas to inland facilities. Using this new technology, a marine shrimp farmer can be located inland, away from sensitive coastal areas. Low salinity ground water is pumped from wells into ponds. Water quality is maintained in a closed system; using environmentally friendly, zero-discharge management by recycling water in settling ponds and aeration of production ponds. Based on these significant advances, it is clear that inland low salinity culture of marine shrimp is viable and should be possible in Puerto Rico if an appropriate inland low salinity ground water aquifer can be utilized.

1.3 Water Resources in the Lajas Valley

In 1955, an extensive irrigation and drainage system was completed by the Puerto Rico Water Resources Authority in the Lajas Valley. This drained wetland areas of the Valley including two lagoons near Guanica. A third lagoon in the Lajas Valley (Cartagena) was maintained as a wildlife refuge and is currently managed by the U.S.F.W.S. A gravity flow cement canal system brought fresh water down from Lago Loco reservoir (a surface water resource) for irrigation to areas limited to north of the principal drainage canal. As such, not all sections of the Lajas Valley receive fresh water from this aging infrastructure. Because of the inefficient manner that water is delivered to area farms and the many broken sections of canal, close to half the water released from the reservoir is lost before reaching Valley farmers. In 2005 the actual demand (amount sent) for agriculture in the Lajas Valley was 3 388.56 million gal/y (12 825.7 million L/y) while the theoretical demand (farms need) was 1 827.56 million gal/y (6 917.3 million L/y) (Instituto de Investigaciones sobre Recursos Agua y el Ambiente de Puerto Rico 2005). The difference between the two values is due to losses in the distribution system. So for every 1.0 gallon (3.785 L) needed by a farmer, 1.9 gallons (7.02 L) must be withdrawn from the reservoir just to compensate for evaporation and loss. In addition, during 2006, diversion from Lago Loco to the Valle de Lajas Irrigation District provided the surface water withdrawn by the PRASA to filtration plants in Sabana Grande (Maginas filtration plant), in Lajas (Lajas filtration plant) and in Cabo Rojo (Betances filtration plant). Public-supply water withdrawals from the Valle de Lajas Irrigation Canal represents about 56% of the total withdrawals from the Lago Loco reservoir to the Lajas Valley Irrigation District (Molina, 2008). Lastly, irrigation water is also limited by the loss of storage in the reservoir due to sedimentation. According to (Soler-López 2000) sedimentation in Lago Loco has reduced its storage capacity from 2.4 Million m^3 in 1951 to 0.87 Million m^3 in 2000 which represents a 64% loss. Consequently, a significant limiting factor in development of the Lajas Valley agricultural reserve is limited fresh water available for irrigation.

Fresh ground water is known to occur in the recharge areas of the Lajas Valley (Anderson 1977). This ground water can be utilized to supplement the surface water canals for irrigation due to its low mineral composition. However, for the majority of the Lajas Valley, ground water is brackish (Anderson 1977). Consequently, much of the alluvial aquifer that underlies the agricultural reserve is not suitable for irrigation of traditional crops or drinking water for animals. In 1983, the USGS began a three year comprehensive survey of the ground water resources of the Lajas Valley. This was done in order to evaluate the potential for large scale rice culture and increased industrial development in this area. Since the freshwater irrigation resource is limited by demand for potable water, inefficient and damaged infrastructure, and the ability of the storage reservoir to capture and release water, alternative resources are necessary to supplement any increased demand for fresh water. According to this USGS ground water survey (Graves 1991), the aquifer below the Lajas Valley is brackish in central valley areas, and in 19 cases (out of 20 analyzed for major ions), exceeded potable drinking water standards for dissolved solids and/or Cl concentrations of 500 and 250 mg/L respectively (American Public Health Association et al., 1995). In fact, of the total 160 wells surveyed between 1983 and 1986 (Figure A.2), only 38 were in use. Many were simply abandoned due to high Cl concentrations. Graves (1991) described the ground water aquifer below the valley floor as a “nonhomogenous, antistrophic confined aquifer comprised of alluvial deposits of Quaternary Age”. The concentration of the major ions varied widely between sites and between different depths at the same site. Generally, Cl concentrations increased with depth.

Since the brackish aquifer can not be used for irrigation or drinking, it potentially could support a shrimp farming industry. Recently, the USGS reported that ground water levels were at record heights during May-June 2006 (http://pr.water.usgs.gov/public/gw/index.html).

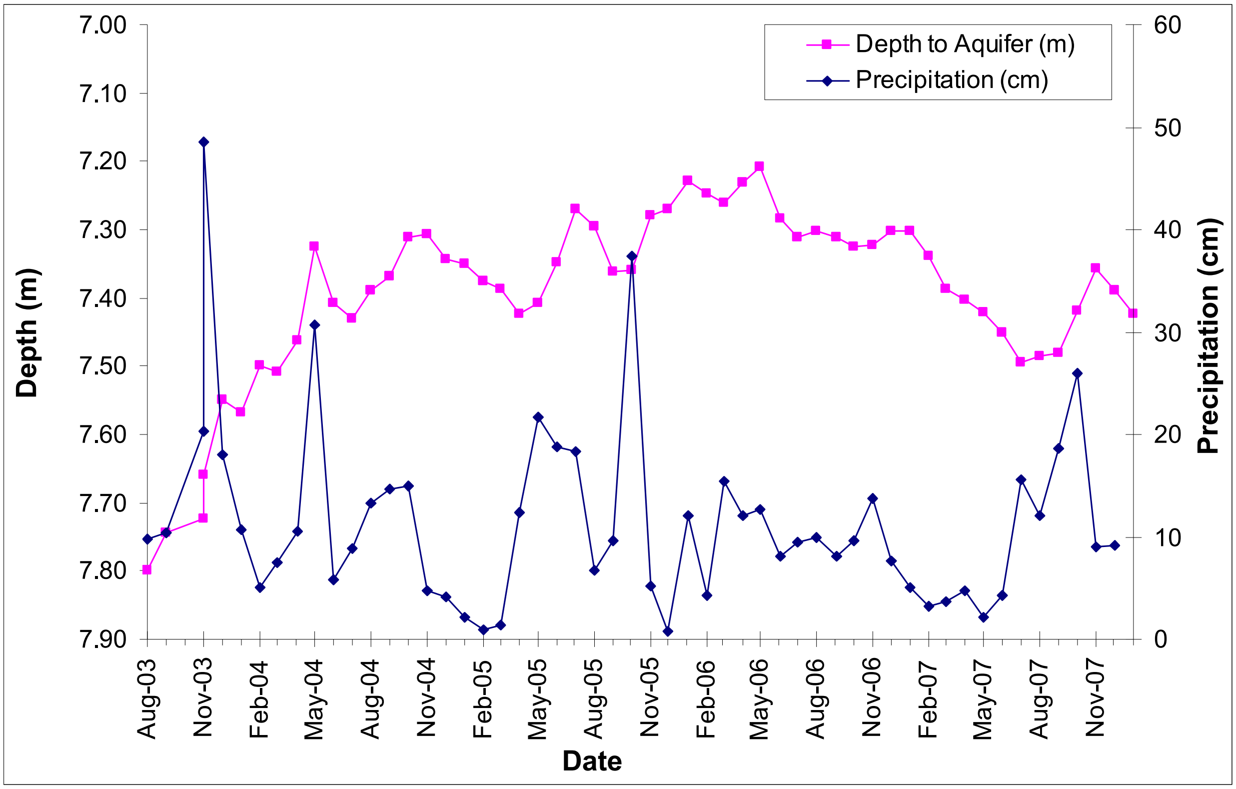


Figure 1.1 Ground water levels and precipitation for the Lajas Experimental Station.

Depth to groundwater measurements within the Valley indicate that localized precipitation has a impact on the near surface aquifer.

Graves (1991) reported daily use of ground water in the Lajas Valley was 2.95 Mgal/d which is 11.17 million L/d or 4.076 billion L/y. More recent (2005) estimates conducted by the USGS in the Lajas Valley indicate that 10.45 million L/d (3.814 billion L/y) were withdrawn from ground-water sources for irrigation purposes (Molina 2008). The reported annual recharge rate is 5.08 cm/y (Anderson 1977), which yields 205 580.3 L/acre/y. The Lajas Valley Agriculture Reserve contains 42,120.6 acres (de la Cruz-Pérez, 2008) which gives us an estimated annual recharge of 8.66 billion L of water for the entire Lajas Valley. Recharge – use yields an estimated 4.846 billion L/y surplus which is currently not utilized. Theoretically, the aquifer could support a 13.277 million L/d increase in use which is more than double the current use.

By definition, one acre-foot of water contains 325 851.4 gal which is 1 233 347.5 L (Anderson 1993). To fill a pond to a depth of 3 feet (0.915 m) requires 3.7 million L/acre. Allowing for a 10% loss/exchange per week (7 days), 370 043 L/week/acre would be required for pond level maintenance. Therefore, 100 acres of shrimp ponds would require about 370 million L to fill the ponds, and 8.88 million L for maintenance; totaling (rounded) 1.258 billion L for the first six months. After harvest, pond water is recycled; thereby reducing the requirement for subsequent pond fills. Due to discharge restrictions (EPA Clean Water Act) inland marine shrimp farms must recycle a large portion of culture water. Treated used water may also be injected back down to the aquifer to further prevent problems associated with aquifer use. In addition, the recommended use of pond liners (which helps protect against contamination of soils) would eliminate seepage and loss would only then be to evaporation. At the Lajas Experimental Station, pan evaporation is estimated at 153 cm/y and rainfall is about 115 cm/y. Since pond evaporation rates are generally estimated by using 0.83 times pan evaporation rates

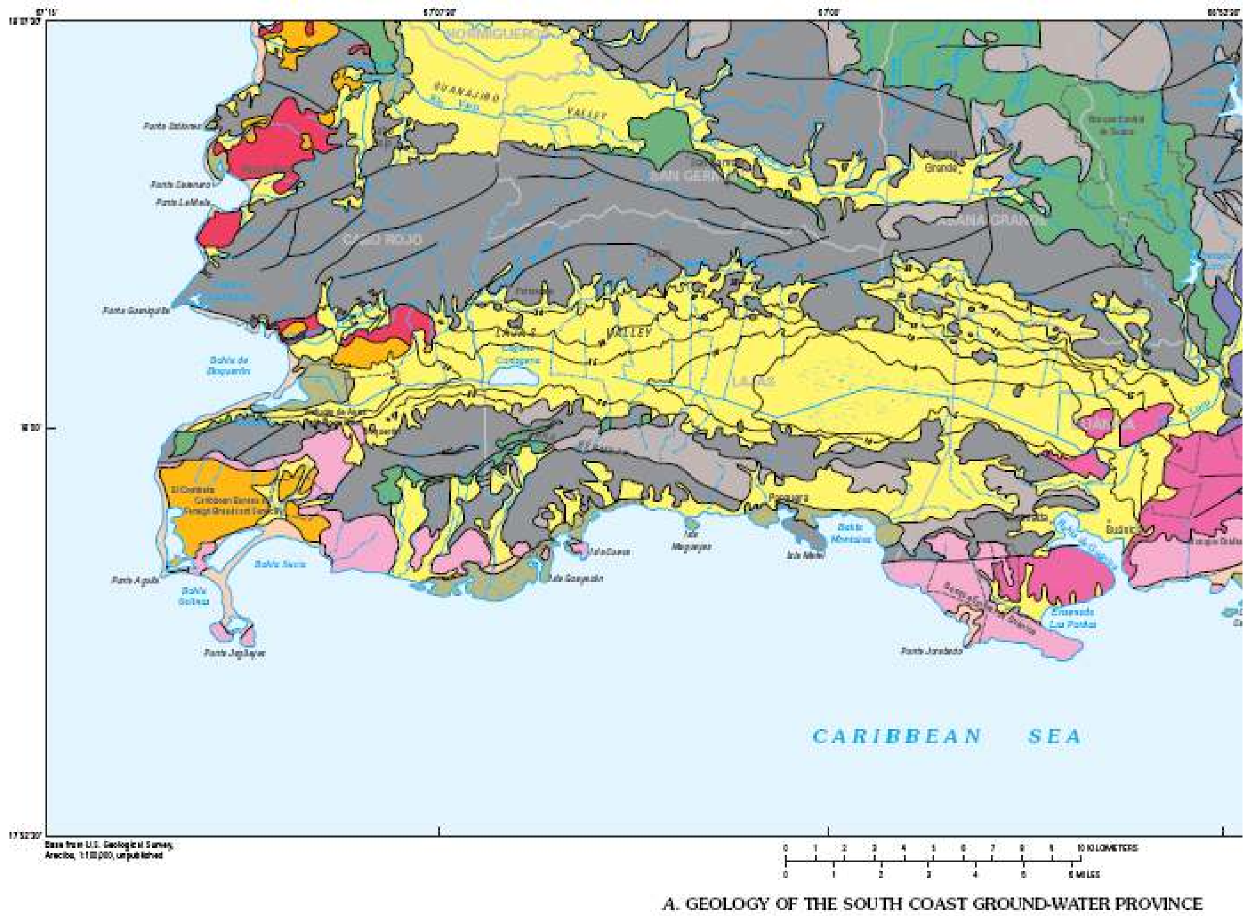
(Boyd 1985) and pan evaporation rates take into account precipitation, 127 cm/y would be the average loss by evaporation. Since one acre-cm of water contains 40 468.6 L, for 100 acres of shrimp ponds 4 046 856.3 L/y would be needed to compensate for evaporation. This estimated maintenance amount of 4.05 million L/y is considerably less than the estimated surplus of 4.846 billion L/y the aquifer receives from natural recharge. This indicates that water requirements for inland shrimp culture at this scale are sustainable and would allow for future development of an inland shrimp farming industry.

1.4 Geology of the study area

Due in part to saline soils (Figures 1.3-1.8) the Lajas Valley's agricultural potential has not been fully achieved through traditional agriculture (Morales 2004, Ramírez 2005). Considering that this reserve is underutilized, sufficiently large land expanses exist that could be used for culturing *L. vannamei*.

Geology and hydrogeology of the area have been surveyed by Graves (1991) and Renken et al. (2002). The Lajas Valley floor is composed of alluvial deposits. Renken et al. (2002) describes the Valley as:

"…having alluvial deposits of 60 to 90 m thick and consist mostly of clayey silt, interspersed with stringers of sand. Deposits of sand and gravel are restricted largely to several small alluvial fans located on the perimeter of the valley. Clayey silt underlying the central part of the valley is distal fan or lacustrine in origin. However, the distribution and character of bedrock units that underlie the clayey alluvium of the central valley is poorly known, largely due to scattered well control. Limestone strata that represent potential aquifers underlie the central part of the valley and are possibly equivalent to irregularly-distributed sequences of Cretaceous limestone strata exposed along nearby foothills. West of Laguna Cartagena, clayey alluvium overlies a carbonate-clastic sequence that could be equivalent to nearby outliers of the Juana Díaz Formation and Ponce Limestone…" Figure 1.2.



Deposits of Quaternary age

Beach Deposits

Marsh or Swamp Deposits

Alluvium

Quartz sand deposits **- Tertiary (?) age**

Rocks of Pliocene (?) to Oligocene age

Ponce Limestone

Guanajibo Formation

Juana Diaz Formation (limestone facies)

Juana Diaz Formation (clastic facies)

Intrusive rocks **- Tertiary and Cretaceous age**

Volcanic and sedimentary rocks **- Cretaceous age**

Metamorphic, sedimentary, and igneous rocks **- Early Cretaceous-Late Jurassic age**

— 20 — Contour – Variable altitude (m) of fan-delta, alluvial fan, and alluvial-valley deposits

Fault

Contact

Figure 1.2. Geology of the south coast ground water province (Renken et al., 2002).

The soil of the Lajas Valley is derived from the parent material 'clayey alluvial sediments'. According to (NRCS 2008), there are three predominant soil types in the areas surrounding each selected well (Figures 1.3 - 1.8). They are Guanica clay (GnA), Fraternidad clay (FrA), and Aguirre clay, (AkA) and have the following properties:

GnA
Depth to restrictive feature: More than 203.2 cm
Drainage class: Somewhat poorly drained
Capacity of the most limiting layer to transmit water (Ksat): Very low to moderately low (0.00 – 0.15 cm/h)
Depth to water table: About 0 cm
Frequency of flooding: None
Frequency of ponding: Frequent
Calcium carbonate, Maximum content: 4%
Gypsum, maximum content: 4%
Maximum salinity: Slightly saline to moderately saline (8.0 – 16.0 mmhos/cm)
Sodium adsorption ratio, maximum: 30.0
Available water capacity: Low (about 15 cm)

FrA
Depth to restrictive feature: More than 203.2 cm
Drainage class: Moderately well drained
Capacity of the most limiting layer to transmit water (Ksat): Very low to moderately low (0.00 – 0.03 cm/h)
Depth to water table: More than 203.2 cm
Frequency of flooding: None
Frequency of ponding: None
Calcium carbonate, Maximum content: 4%
Gypsum, maximum content: 2%
Maximum salinity: Nonsaline (0.0 – 2.0 mmhos/cm)
Sodium adsorption ratio, maximum: 2.0
Available water capacity: Moderate (about 22.9 cm)

AkA
Depth to restrictive feature: More than 203.2 cm
Drainage class: Poorly drained
Capacity of the most limiting layer to transmit water (Ksat): Very low to moderately low (0.00 – 0.03 cm/h)
Depth to water table: About 0 – 5 cm
Frequency of flooding: None
Frequency of ponding: Frequent
Calcium carbonate, Maximum content: 15 percent (salinity not reported)
Available water capacity: Very high (about 68.3 cm)

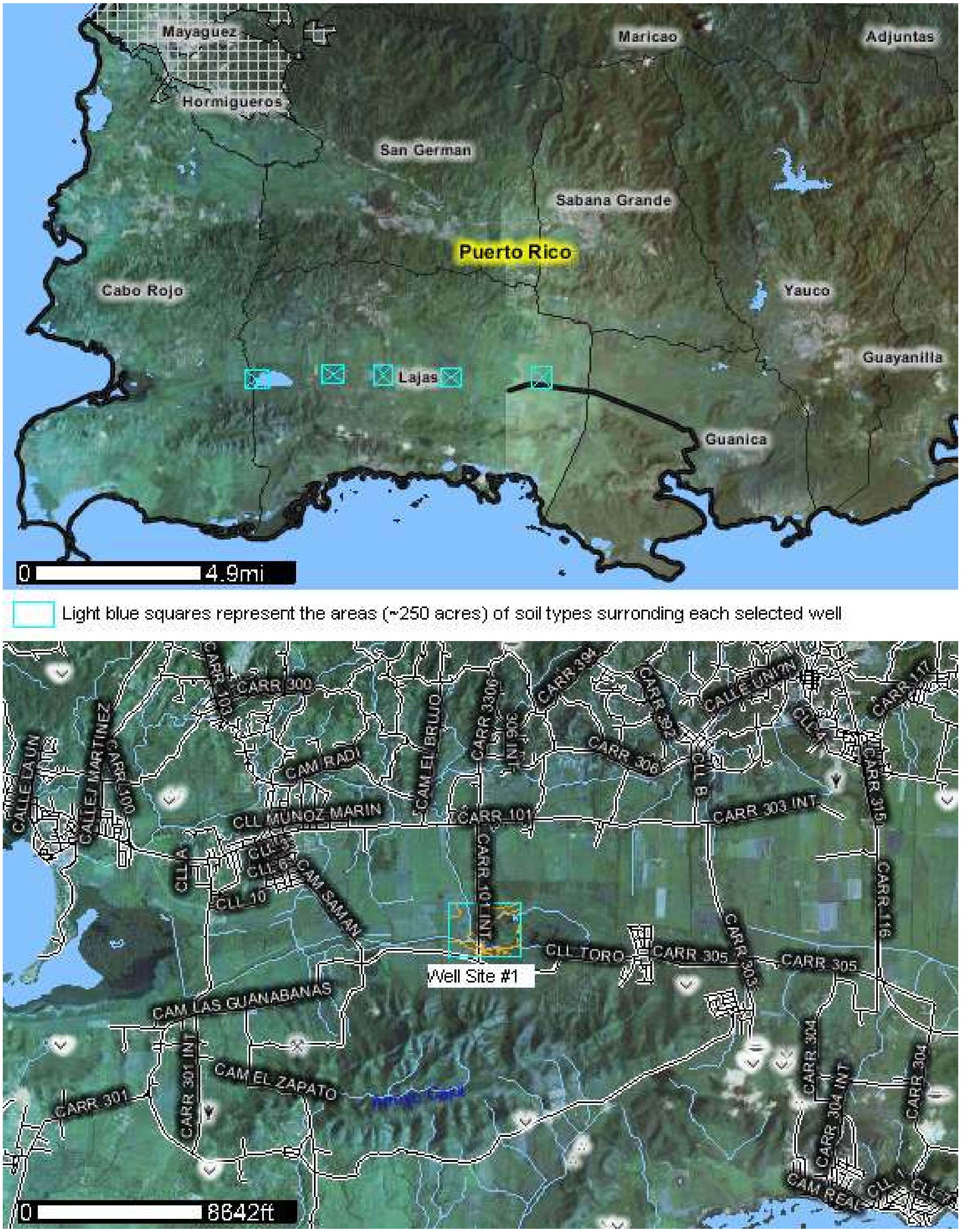


Figure 1.3. Location of five study sites in the Lajas Valley, with close up of site # 1.

According to the U.S.D.A. (NRCS 2008) the soil surrounding the Cartagena well (site #1) is predominantly Guanica clay.

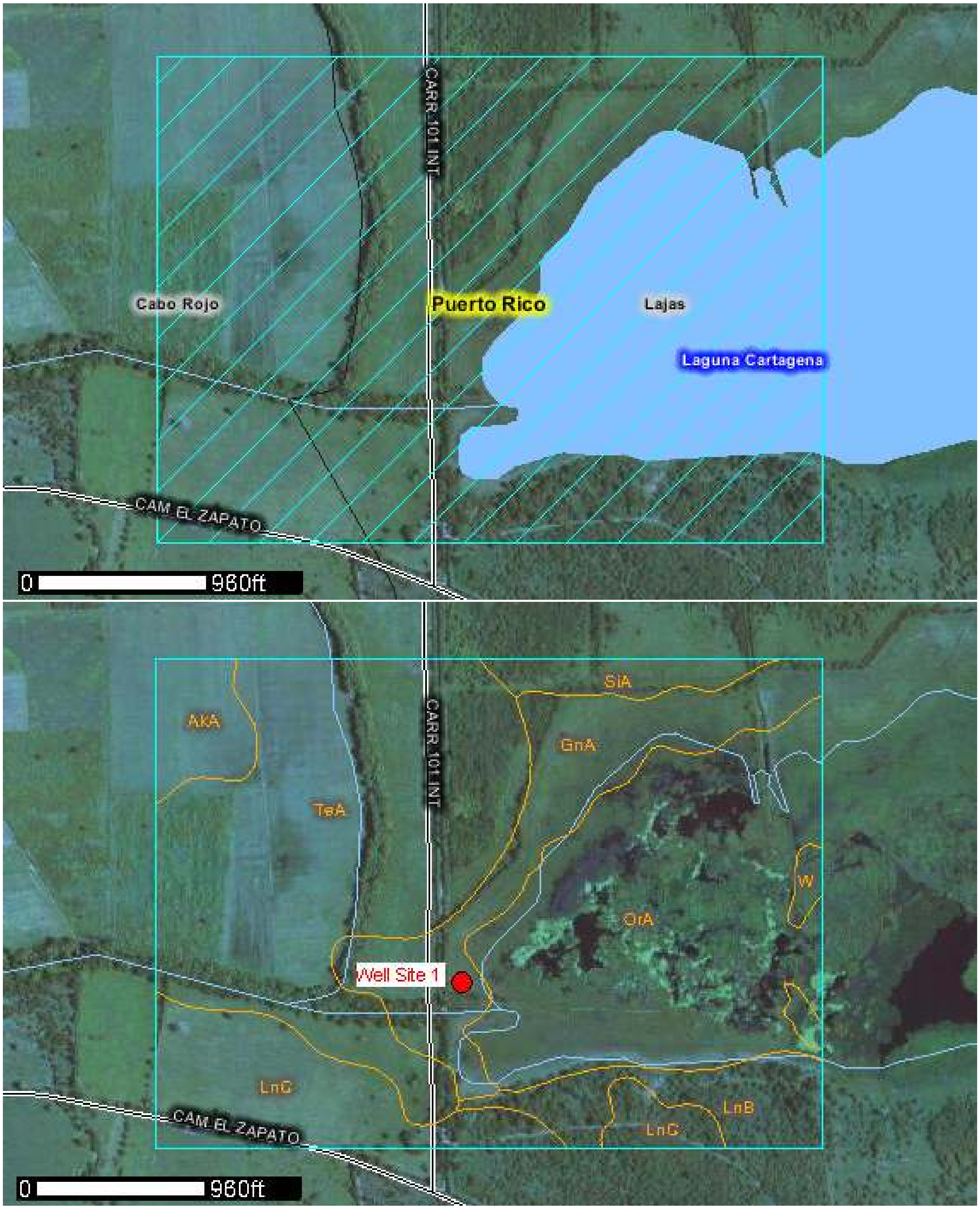


Figure 1.4. Soil types near sample site # 1.

Site #2 (located on the UPR experimental agriculture station) is predominantly Fraternidad clay, and Aguirre clay.

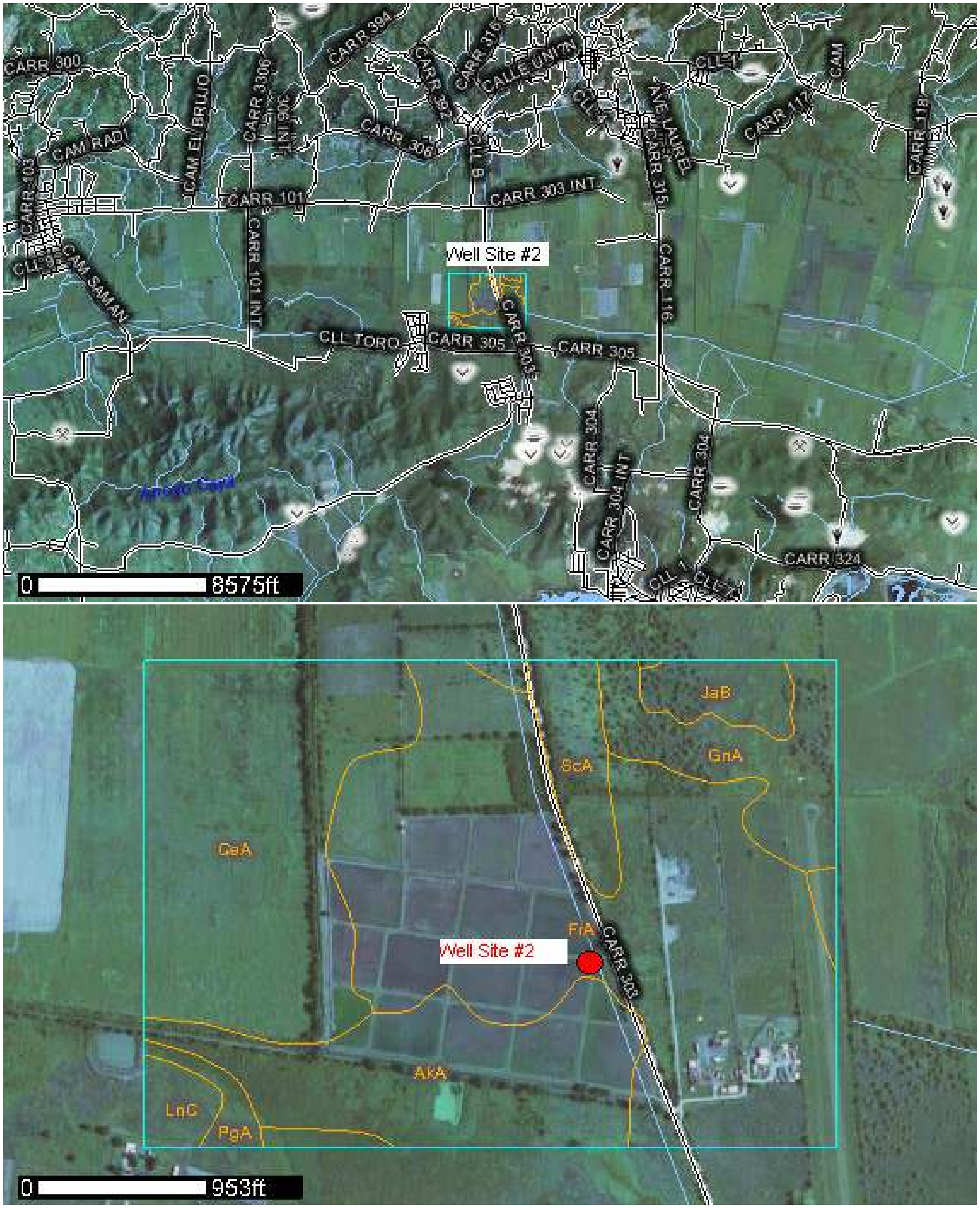


Figure 1.5. Soil types near sample site # 2.

Site #3 (near road 116, Barrio Sabana Yegua) is located on soils of the type 100% Aguirre clay.

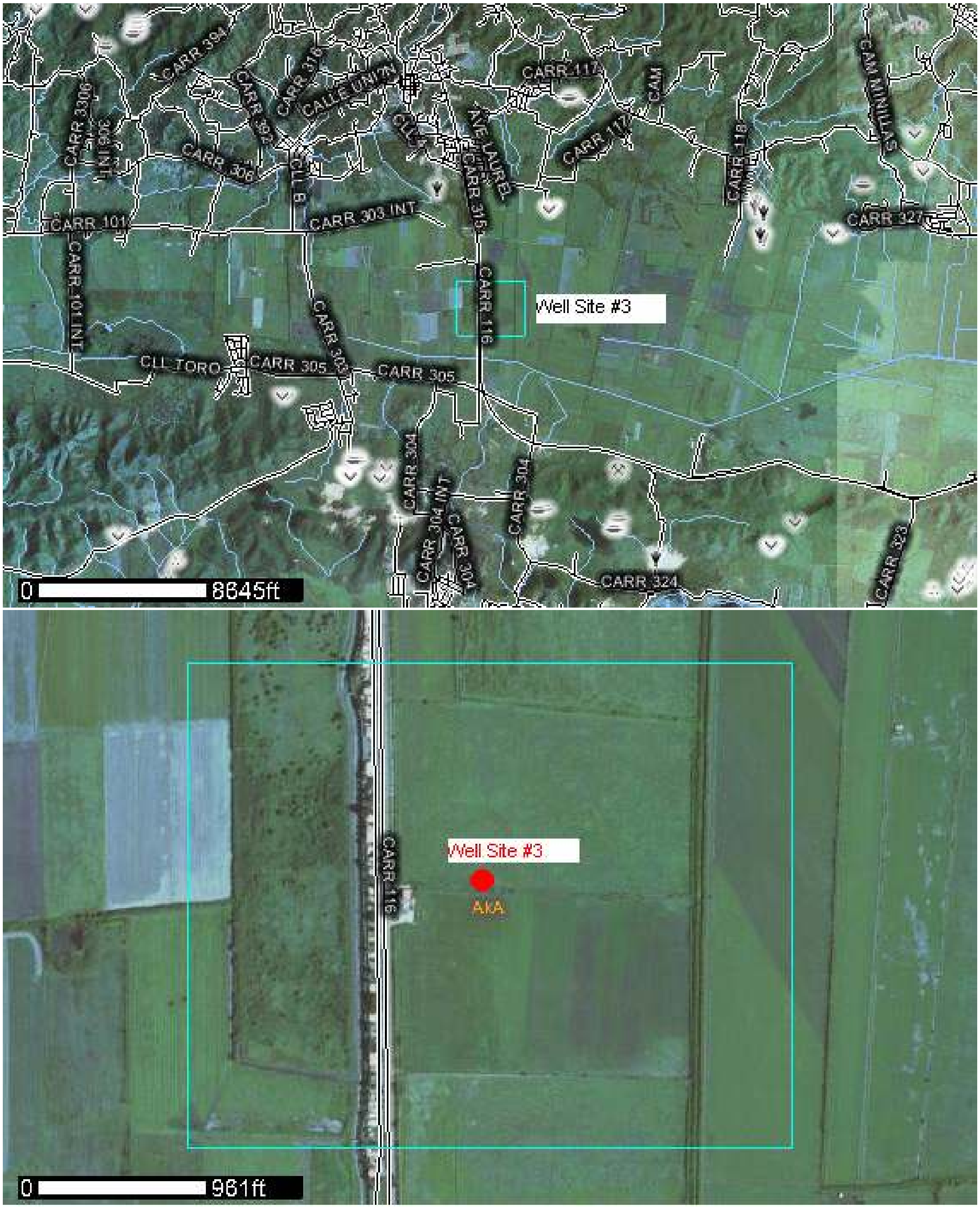


Figure 1.6. Soil types near sample site # 3.

Site #4 (Barrio Lajas Arriba) is also located on Aguirre clay and Guanica clay soils.

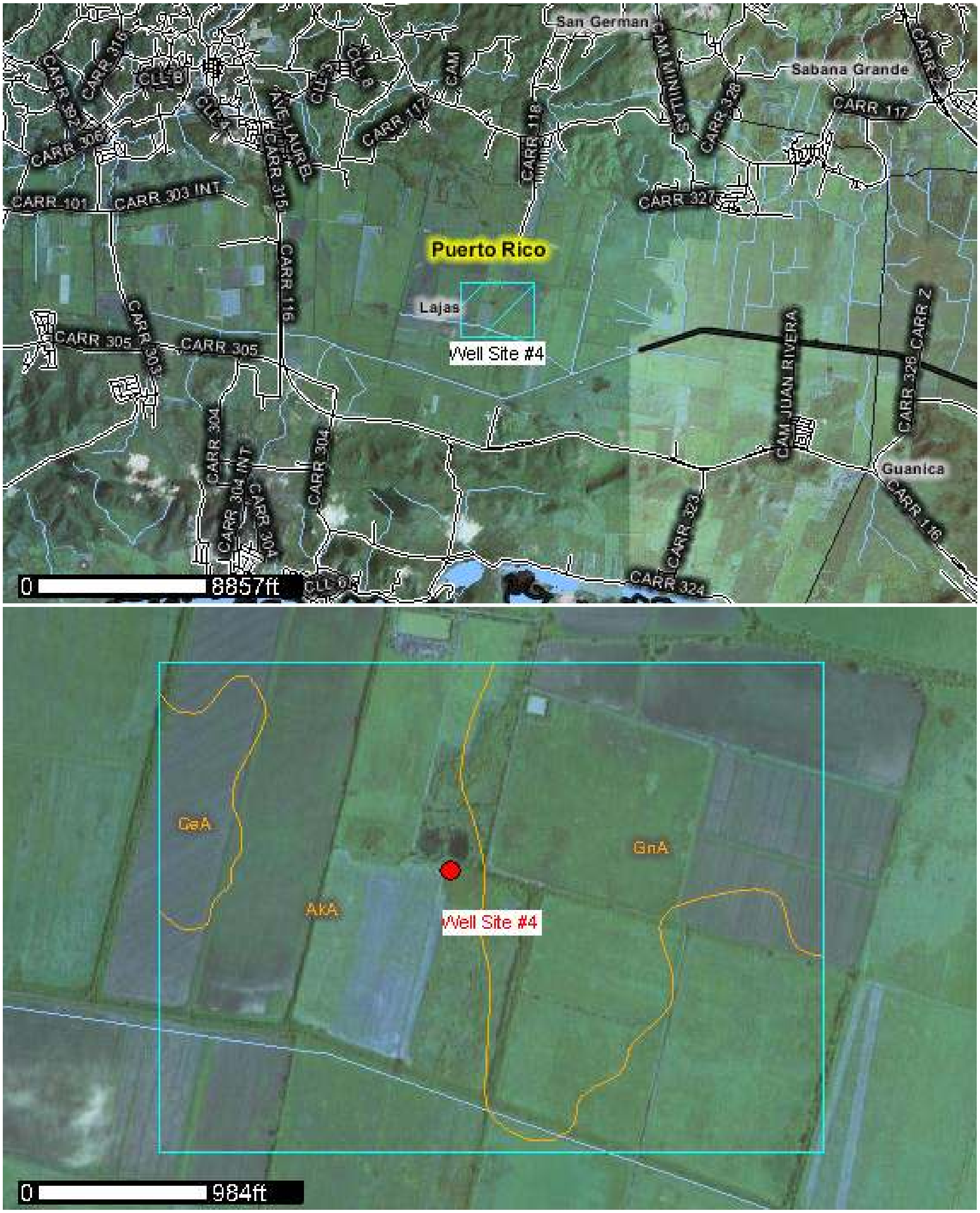


Figure 1.7. Soil types near sample site # 4.

Site #5 (Barrio La Plata) is located on Guanica clay.

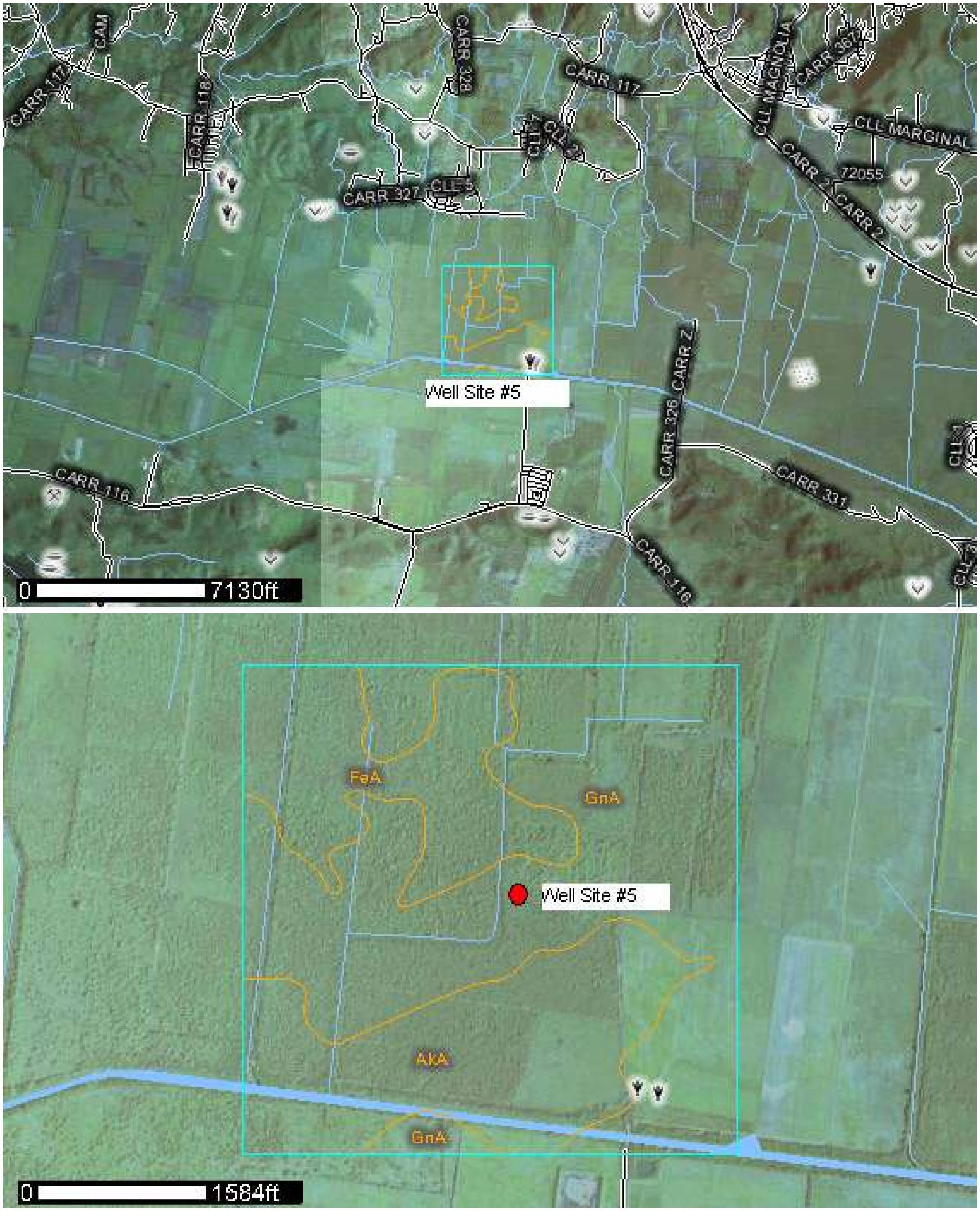


Figure 1.8. Soil types near sample site # 5.

1.5 Summary

The Lajas Valley agricultural reserve is a large (about 42 121 acres, Fig. A3) farming area located in Southwestern Puerto Rico that is protected by law as an agricultural reserve. In view of the worldwide trend towards culturing *L. vannamei* in low salinity ground water and because of reported availability of saline ground water in the Lajas Valley (Graves 1991), trials were initiated evaluating the saline ground water from the Lajas Valley for the production of this marine shrimp. The Lajas Valley was chosen for the study because of favorable environmental conditions, saline aquifer, salt affected soils, and the study area is located within an agricultural reserve.

Objectives

The objectives of this research were:

• Determine if post larval *L. vannamei* can be acclimated to ground water from the Lajas Valley with acceptable survival.

• Determine if water from the saline aquifer is suitable for culture of this marine species by analysis of the following ions and compounds: (Na, Cl, K, Ca, Mg, Mn, CO_3, and SO_4).

• If sufficient ionic concentrations are not present naturally, locate a local source of minerals that can be used to augment any lacking ionic deficiencies.

• Determine the location best suited for this type of culture based on appropriate saline aquifer, marginal soils, and land availability.

If these objectives can be obtained, a new industry in Southwestern Puerto Rico could be developed in areas where traditional agriculture has not been economically viable. Establishing a low salinity marine shrimp aquaculture industry would diversify agriculture production, create jobs, reduce dependence on wild caught species, supplement imports from foreign countries, and also has the potential for exportation which would increase the Gross National Product.

Chapter 2 - Acclimation to Ground Water

2.1 Introduction – Pilot Study

Because *L. vannamei* is a marine species, it is very important to consider the salinity of water used in culturing the shrimp. Any time you try to maintain an organism in environmental conditions outside its natural range, mortality is likely. Fortunately, because *L. vannamei* naturally lives part of its life in fluctuating salinities, acclimation to inland low salinity water is possible. However, due to drastic osmotic differences between seawater at 35 ppt and well water at 1-10 ppt, acclimation must be carefully accomplished. Since Mendes and Pedreschi (1998) reported that *L. vannamei* could be acclimated to fresh water with salinity as low as 0.09 ppt, trials using water reported to be 'salty' were attempted.

From previous experiments (Mace unpublished data) it is known that *L. vannamei* can not be cultured in some well waters from the Lajas Valley. During 2002, several hundred shrimp were purchased from Eureka Marine Inc, in Dorado, PR. These shrimp were transported to the UPR Department of Marine Science Magueyes Island research facility, and placed into 76 L aquariums at a density of ten PL per tank. Water in these aquaria was full strength sea water (35 ppt). Previously conditioned sponge bio-filters were added to each aquarium to maintain water quality. Shrimp were fed to satiation twice daily with live artemia and a commercially available fry powder (50% protein) during the trial. Uneaten feed and fecal material was removed daily during the experiment. McGraw et al. (2002) reported that PL must be at least 15 d old before they can be acclimatized to low salinity water. These shrimp were only 8 d old when delivered to the lab, so they were held for one week at full salinity (35 ppt). Low salinity acclimation began once the PL were 15 d old. Three wells (Caribe Fisheries Inc., Finca Rigau, and Western

Hay Farm) were chosen from Lajas Valley farms whose owners expressed interest in low salinity shrimp culture. Caribe Fisheries Inc. is a tropical aquaculture farm located in Barrio La Plata. Finca Rigau, located in Sabana Grande, is part of the old Langostinos del Caribe fresh water prawn facility which was divided up and sold after it closed. Western Hay Farm Inc. is one of the Lajas Valley's largest land owning companies with properties through out the Lajas Valley. Well water from the Western Hay Farm came from a domestic well at Francis A. Pérez Riveiro's farm in Barrio La Plata. The owners of these wells reported that the water was salty, and not good for drinking. All three water samples tested *in situ* at approximately 1 ppt salinity using a Coral Life brand specific gravity meter. A LaMotte Freshwater Aquaculture Kit was used to test for the following parameters: NH_4 nitrogen, NO_2 nitrogen, temperature, and chlorides. Samples from each well were transported to Magueyes Island and low salinity acclimation began with the lowering of water levels in aquaria, and gradual drip addition of well water. This process was undertaken over a period of 3 days. Salinity was lowered by half over the first 24 h, then by half again the second 24 h. On the third and final day of salinity acclimation, water was siphoned out of aquaria and well water was dripped in until endpoint salinity was the same as original well water salinity +/- 1 ppt. Shrimp were observed over the 72 h low salinity acclimation experiment.

Results were not favorable for low salinity acclimation using these three wells. Survival was 0% for all three well sources after 72 h. NH_4 and NO_2 Nitrogen were negligible due to the effective bio-filtration and frequent water changes. Temperature fluctuated between 21-23^{o}C. Cl ion was the following: Caribe Fisheries (180 ppm), Finca Rigau (160 ppm), and Western Hay Farm (165 ppm).

Conclusions from this small scale pilot study indicate that well water from these selected areas in the Lajas Valley is not suitable for the culture if marine shrimp. Water quality (in terms of ionic concentrations) from aquifers is more important than total salinity. In addition, low cost salinity meters commercially available for aquarium use are probably not reliable enough to be used for this purpose on salinities less than 1 ppt. Later that year Whetstone et al. (2002) published the following information specifying minimum ionic concentrations required for low salinity water intended for culturing *L. vannamei* (Figure 2.1).

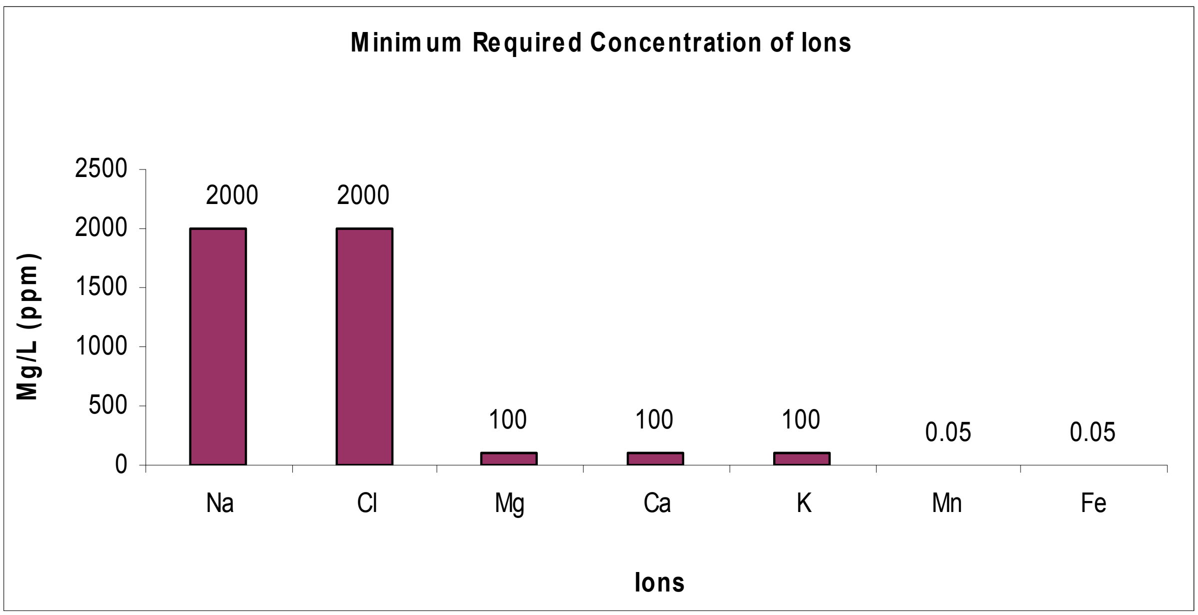


Figure 2.1. Minimum ionic concentrations required for low salinity shrimp culture water.

From this data it was concluded that none of the selected wells had sufficient Cl ion for successful shrimp acclimation. Apparently, water from these wells is too fresh for this species to survive. However, during this period both Caribe Fisheries and Finca Rigau had ongoing experiments using a mixture of seawater and well water. Data from these trials showed that shrimp could be cultured under diluted seawater conditions, with salinities down to 1 ppt. This also supports the conclusion that the ionic ratios may indeed be more important than over all salinity. Further experiments should utilize water with greater concentrations of the major dissolved ions found in seawater (Na, Cl, K, Mg, Ca) as indicated by McGraw and Scarpa (2002). Fortunately, a USGS report (Graves 1991) is available on the Lajas Valley ground water. Using this report as a guide, other wells were discovered and used in experiments of this nature.

2.2 Materials and Methods - Site Selection

Five well sites were selected in the Lajas Valley based on salinities (ranging from 0.7-9.8 ppt) and locations (spanning the Lajas municipality) as reported in Graves (1991).

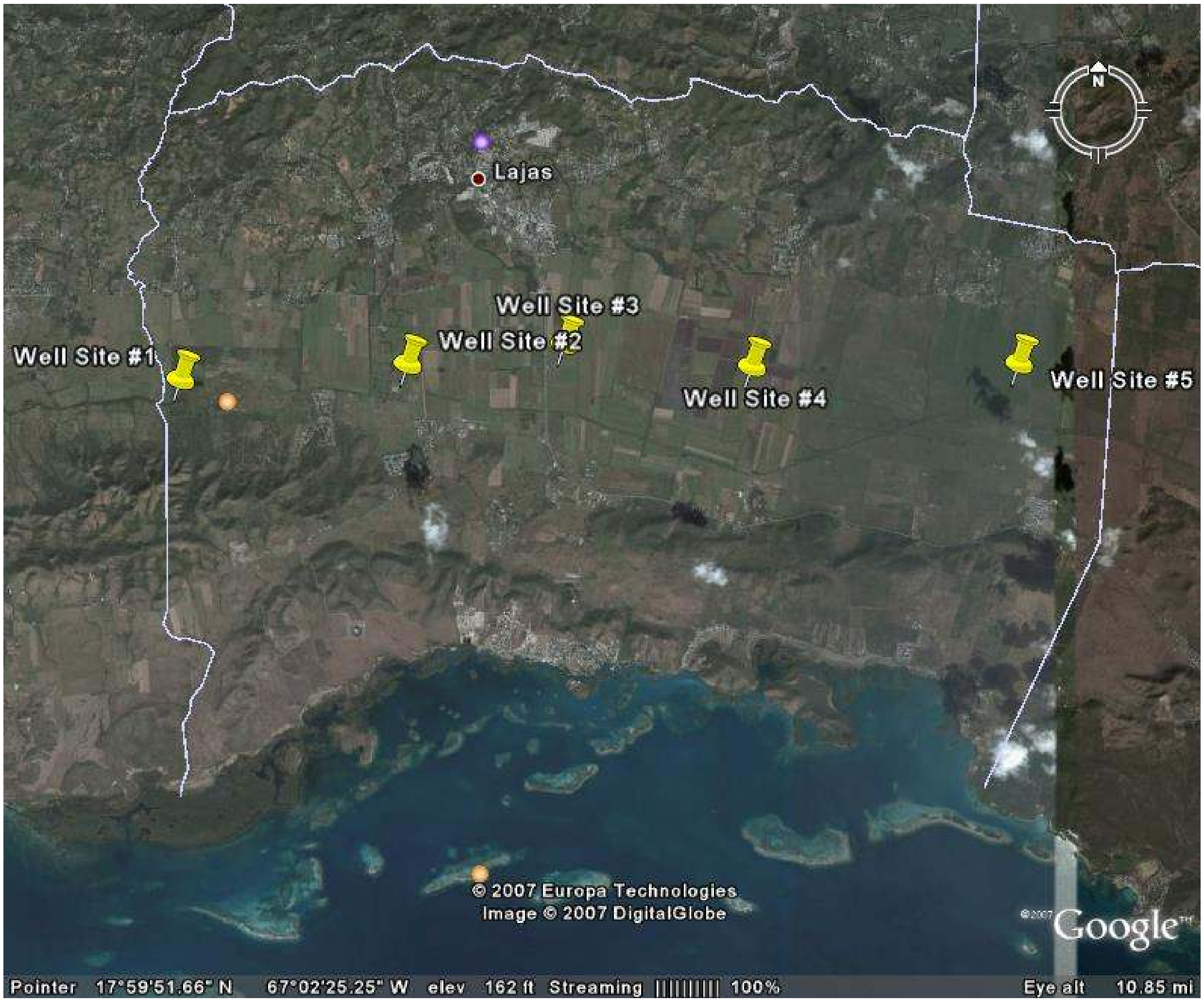


Figure 2.2. Google Earth image indicating locations of wells sampled in Lajas, Puerto Rico.

Landowners were contacted for permission to sample, and wells were purged following standard protocols (Nielsen and Nielsen 2006) by pumping (12-volt twister well pump) and discarding three times the volume of the well casing using the formula (well depth (ft) from ground – depth to water) x .653 = Casing Volume (gal) x 3.785412 = L. Following purging, samples were collected following protocols specified by Pace Analytical Inc. Samples slated for metal analysis were preserved following standard protocols (EPA 1983) with nitric acid. Samples were chilled with ice, and delivered to Pace Analytical Inc. in San German for chemical analysis. The following ion concentrations were determined and compared to standard seawater: Na, Cl, K, Mg, Ca, SO_4, CO_3, and the trace element Mn. Three of these well sites (1, 3, and 5) are piezometer test well batteries (multiple wells). Piezometers are scientific test wells specifically designed to sample water from a known depth or aquifer. They are generally constructed of 10 cm PVC pipe, and perforated or 'screened' at a specific known depth range. This limited area of perforation allows water from only that depth to enter the well casing and be withdrawn for testing. Usually, they are low production wells and not appropriate for general use as little water can be extracted from them. Well sites 1, 3, and 5 are piezometers, constructed by the USGS between 1983-1986, for the ground water survey (Graves 1991). In contrast, production wells tend to have much larger screened areas, allowing for a greater quantity of water to enter the casing. There are 10 wells at the Cartagena site # 1, with depths ranging from surface to 25 m. The deepest wells were selected at each of the sites with multiple wells to sample the deep aquifer that is not affected by evapotranspiration. According to Graves (1991), shallow test wells between surface and 16 m reflected a concentrating effect of evapotranspiration resulting in higher chlorides at shallow depths. Chlorides generally decreased with depth to 16 m then increased with depth subsequently. This was attributable to connate (old, and rich in minerals) water present in the deeper aquifer, which has not been flushed out. Since we are interested in relatively high salinity ground water for marine shrimp culture, the deepest wells were selected as they should be higher in salinity and mineral content and more stable over time.

Well site # 1 is located in the Cartagena Lagoon Wildlife Refuge area, near the western boundary, with approximate Latitude / Longitude 18°00'55"N / 067°06'33"W. Depth to water is 3 m. Reported well depth is 25 m and the well casing is screened (perforated) between 24.3-25 m, which allows only water from this depth to enter the casing. Access is by dirt road near the western boundary of the U.S. Fish and Wildlife Refuge. Recently, a water retention dam was constructed causing flooding of the well site during the rainy season of 2005. Originally this site had appropriate characteristics for this study. However, due to the flooding of the Cartagena Lagoon, access to the well was compromised. In addition, water production from this well is very low. Recharge is on the order of months, making it difficult to extract sufficient quantities of water. Draw down reaches the bottom of the well, and water quality (turbidity increases) and quantity is adversely affected as pumping empties the well casing. Salinity was 0.7 ppt. This was the lowest salinity well site used for this study. Further studies from this site are not anticipated.


Figure 2.3. Well site #1 is located on the Cartagena U.S.F.W.S. Refuge.

Well site # 2 is located on the UPRM Lajas Experimental Agricultural Station, south of the UPR Dairy, next to road 303. Approximate location (Latitude / Longitude) is 18^{o}01'03"N / 067^{o}04'25"W. Depth to water is 3 m, overall well depth is reported as 64.6 m, and the casing is screened between depths 27.4-51.8 m. Access is easy due to the very near location of a paved road. This is also a test well constructed by the USGS, but it was designed as a production test well. The well casing has been perforated over a depth range exceeding 24 m which allows a greater amount of water to enter the well casing from a larger portion of the aquifer. Consequently, pumping doesn't affect turbidity of the water and draw down is insignificant. Water from this well is clear and pumped as much as needed. This was an excellent well site used throughout the study. Salinity was 6.7 ppt. Future studies of this nature are recommended to include this site.


Figure 2.4 Well site # 2 is located on the UPR-RUM Experimental Agriculture Station.

Well site # 3 is located in a cow pen on the edge of a hay field east of road 116 in Lajas, behind Irizarry Rentals in Barrio Sabana Yeguas. Approximate location (Latitude / Longitude) is 18°01'11"N / 067°02'52"W. This is a battery of 5 piezometer wells with the deepest having a depth to water of 7.6 m. Overall well depth is reported as 75.3 m but it was only screened between 61-62 m and has collapsed below this depth. Access is relatively easy due to the close location of a paved road. Water from this well is clean as turbidity is not affected by pumping. However, water production is very low. Recharge is on the order of months, making it difficult extract a sufficient amount of water, consequently this well site is not recommended to be included in future studies of this nature. Salinity was 7.7 ppt.


Figure 2.5. Well site # 3 is located near road 116 on a hay farm.

Well site # 4 is located on a hay farm, south on road 322 interior, in barrio Lajas Arriba. Approximate location (Latitude / Longitude) is 18°01’00”N / 067°01’06”W. Depth to water is 2 m. Overall well depth is reported as 52.4 m with the casing screened between depths 22.6-47 m. As such, this test well is considered a production well and not a piezometer. Access is by four-wheel drive vehicle only during dry periods on roads used by tractors to cut and harvest hay. Water clarity is excellent as pumping does not affect turbidity. Because this is a production well, water flows freely, with draw down not affecting availability of water. This was the highest salinity well reported in the USGS survey with a salinity of 9.8 ppt (Graves 1991). Future studies could utilize this well site. However, the condition of the access road must be taken into account as during the rainy season it is not passable by automobile. The well is also difficult to find as vegetation will conceal the well casing.

Figure 2.6. Well Site # 4 is located on a hay farm in Barrio Lajas Arriba.

Well site # 5 is located in barrio La Plata, south of road 327 interior. This is the third piezometer well battery constructed by the USGS for their study (Graves 1991). There are 5 wells at this site. One well is artesian and flows freely. Approximate location (Latitude / Longitude) is 18^{o}01’03”N / 066^{o}58’35”W. The deepest well has the following characteristics: depth to water is 3.1 m, while overall well depth is reported as 75.3 m with the casing screened between 48.2-50.6 m. Water clarity is good initially, but pumping substantially increased turbidity. Access is by four-wheel drive during dry periods only due to the condition of the dirt road. During the dry season, the dirt road connects La Plata and Cuesta Blanca, connecting road 116 with road 327. Salinity is 3.5 ppt. This well site could be used in future studies, but access is compromised during the rainy season as the road is not passable by automobile. This area is the most promising area for the construction of a low salinity inland marine shrimp demonstration facility due to its saline soils, underutilized land, and promising aquifer.

Figure 2.7. Well site # 5 is located in Barrio La Plata, south on road 327 interior.

The graphic below (Figure 2.8) is a diagram of well depths for the five wells selected in the Lajas Valley. The lower section of each well casing is screened to allow water to enter the well. Wells 1, 3, and 5 are the piezometer test wells while wells 2 and 4 are production test wells. The obvious difference is the length of the screened sections. Production wells are screened over a much larger section allowing for water to enter the well casing at a much higher flow rate while piezometers are designed to pull water from a very specific aquifer depth.

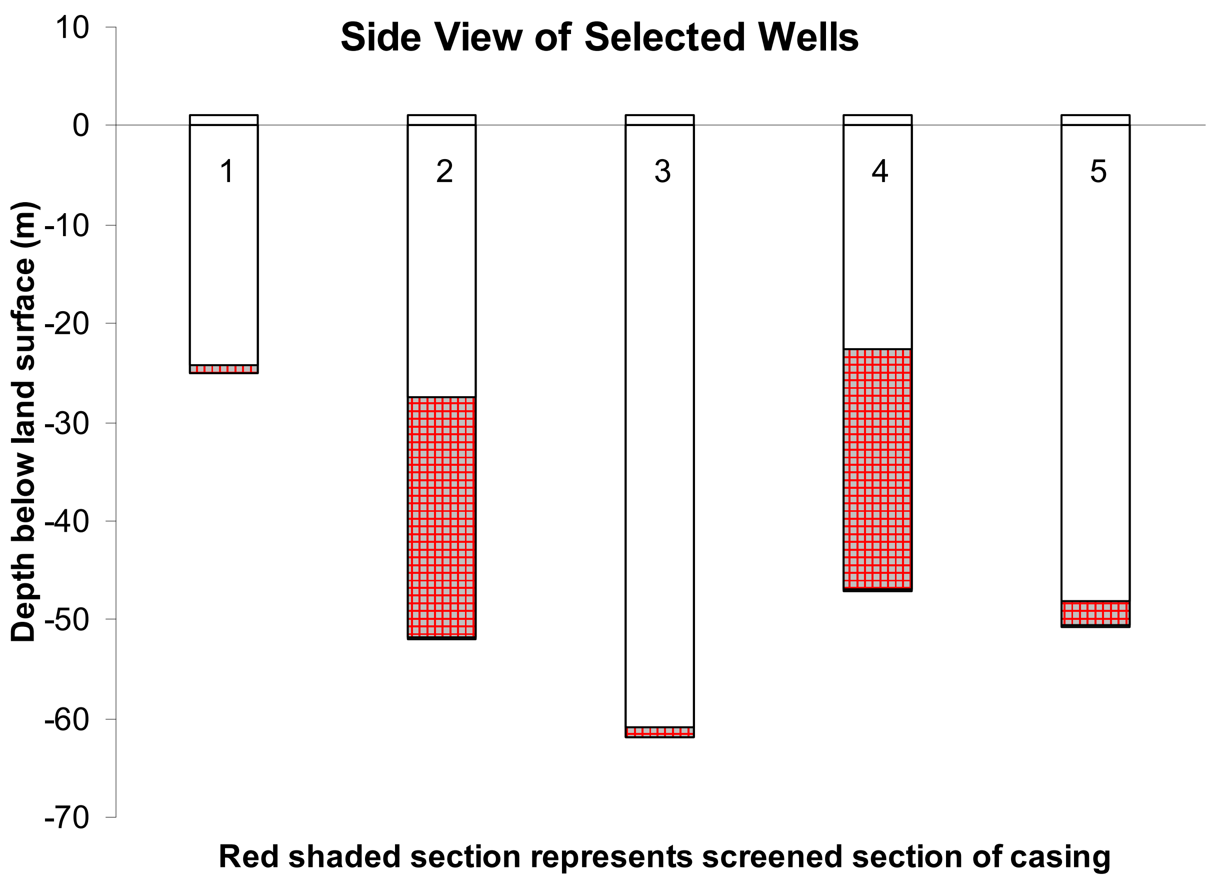


Figure 2.8. Well depths and screened sections of the five selected wells.

2.3 Materials and Methods - Acclimation Study

Post larval *L. vannamei* were used for the acclimation bioassay. Specific pathogen free (free from the following viruses: IHHNV, TSV, BV, NHP, YHV, and WSSV) seven-day-old post larval *L. vannamei* (PL_7) were imported from Florida by Eureka Marine (a shrimp farm located in Dorado, PR). The PL were then sold to us and held for 11 days in artificial sea water (35 ppt salinity) at Caribe Fisheries Inc. (a commercial fish farm located in the Lajas Valley). Acclimation trials were initiated after the PL reached day 18. Salinities were measured with an Aquatic Ecosystems portable refractometer (+/- 1 ppt), or an Oaktron digital salinity meter (+/- 0.1 ppt). The digital salinity meter was most accurate at salinities 10 ppt or less where as the refractometer was used predominantly for salinities over 10 ppt. Both meters were provided by Caribe Fisheries Inc., and are useful for estimating salinities for aquaculture purposes. It is doubtful that either meter was as accurate as expensive laboratory meters are. Budget restraints prevented the purchase of such a meter, so reported salinities are considered estimates. More importantly, the ionic concentrations (which were measured by a laboratory) are most critical to have accurate values.

The experimental design used for the following acclimation bioassay was set up in order to test for significance with a completely randomized ANOVA. Three replicates of each trial were incorporated. Aquariums were stacked on a rack that allowed each tank to have another below it. In Figure 2.9, each small square indicates a 76 L aquarium. Since three replicates were used for each site including the control, there were 18 tanks on each level, totaling 36 aquariums. The upper aquariums held the sample well water. The lower aquariums held a small amount of artificial sea water and 50 shrimp each. Water was gradually dripped from the upper tanks to the lower tanks, in order to dilute the artificial sea water slowly.

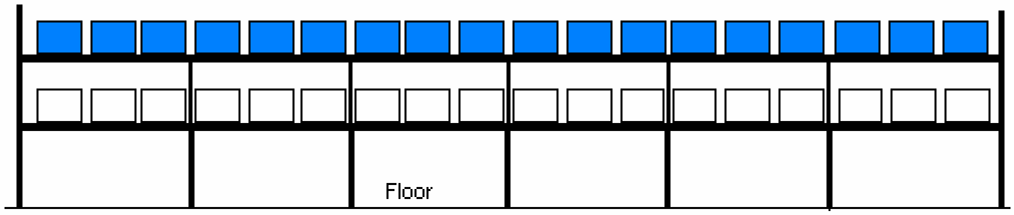


Figure 2.9. Aquarium rack showing experimental design.

Eventually all the aquariums on the bottom level were filled with pure well water (except for the control which was artificial sea water diluted to 15 ppt) in the following manner.

Three 76 L aquaria (randomly distributed on the rack) were filled with water from each of the 5 well sites, or the control. Identical aquaria were placed directly below these tanks. Exactly 7.6 L of artificial seawater (35 ppt) was placed in each empty aquarium on the bottom row. Fifty PL were placed in each aquarium with artificial seawater and aeration. Low salinity water was slowly added at approximately 7.6 L/h, for 8 h, until the lower aquariums were nearly full and salinities were reduced (Figure 2.1). Salinity was therefore reduced over an 8 h period during the afternoon. Salinities stabilized by 7 p.m., and no water was added between 7 p.m. and 7 a.m. the following day. Salinity was not changed over night. The following morning, water was then siphoned out by hose until just enough water was left to cover the shrimp and prevent stress from drying out. This was done to remove as much of the artificial seawater as possible. Well water was then added into the aquaria by gravity, salinity was further reduced until source water salinity end point was achieved to within 0.1 ppt, which took no more than 6 h. All aquaria were at their source water endpoint by 28 h. Shrimp were observed for 48 h and survival was recorded. Live artemia was fed twice daily (at 7 am and 6 pm) during the trial.

2.4 Results

The following ionic concentrations were determined between 1983-1986 for Graves (1991) and in 2005 by laboratory analysis at Pace Analytical Inc., San German, PR for samples from wells 1–5 (Table 2.1).

Well Location Name	Well # 1 Cartagena		Well # 2 UPR-RUM		Well # 3 Road 116		Well # 4 Bo. Arriba		Well # 5 Bo. La Plata	
Data Source	Graves	Pace	Graves	Pace	Graves	Pace	Graves	Pace	Graves	Pace
Well I.D. #	150	1	138	2	155	3	139	4	156	5
Depth to screen (m)	25	25	51.8	51.8	62	62	47	47	50.6	50.6
Specific Conductance (uS/cm)	n/a	n/a	10600	n/a	15400	n/a	19300	n/a	4860	n/a
Temperature C	n/a	n/a	26	n/a	26	n/a	28	n/a	29.5	n/a
Salinity (ppt)*	n/a	0.7	5.9	6.7	8.8	7.7	10.6	9.8	2.4	3.5
Na (mg/L)	n/a	110	1500	1800	1900	2200	3500	3300	830	900
Cl (mg/L)	n/a	78.4	3200	3530	5800	5700	6400	6920	1100	1270
K (mg/L)	n/a	3.9	3.3	3.5	5.7	6	3.9	4.3	3.6	3.8
Mg (mg/L)	n/a	21	500	560	680	500	590	290	67	75
Ca (mg/L)	n/a	17	280	260	780	740	600	510	140	150
Mn (mg/L)	n/a	1.2	0.06	0.16	5.4	12	0.06	0.042	0.84	0.9
SO_4 (mg/L)	n/a	21.1	1300	1410	1100	729	2100	2470	500	483
CO_3 (mg/L)	n/a	0.41	n/a	0.62	n/a	0.34	n/a	0.24	n/a	0.77
Alkalinity (mg/L $CaCO_3$)	n/a	n/a	544	n/a	263	n/a	315	n/a	431	n/a
Hardness (mg/L $CaCO_3$)	n/a	n/a	2782	n/a	4747	n/a	3926	n/a	625	n/a
pH	n/a	n/a	7.3	n/a	7.3	n/a	7	n/a	7.1	n/a

Table 2.1 Water quality data from 1991 and 2005 for selected wells in the Lajas Valley.
* Note: Estimates for salinity were calculated for Graves by the mathematical relationship between specific conductance and temperature. For Pace, a digital salinity meter was used.

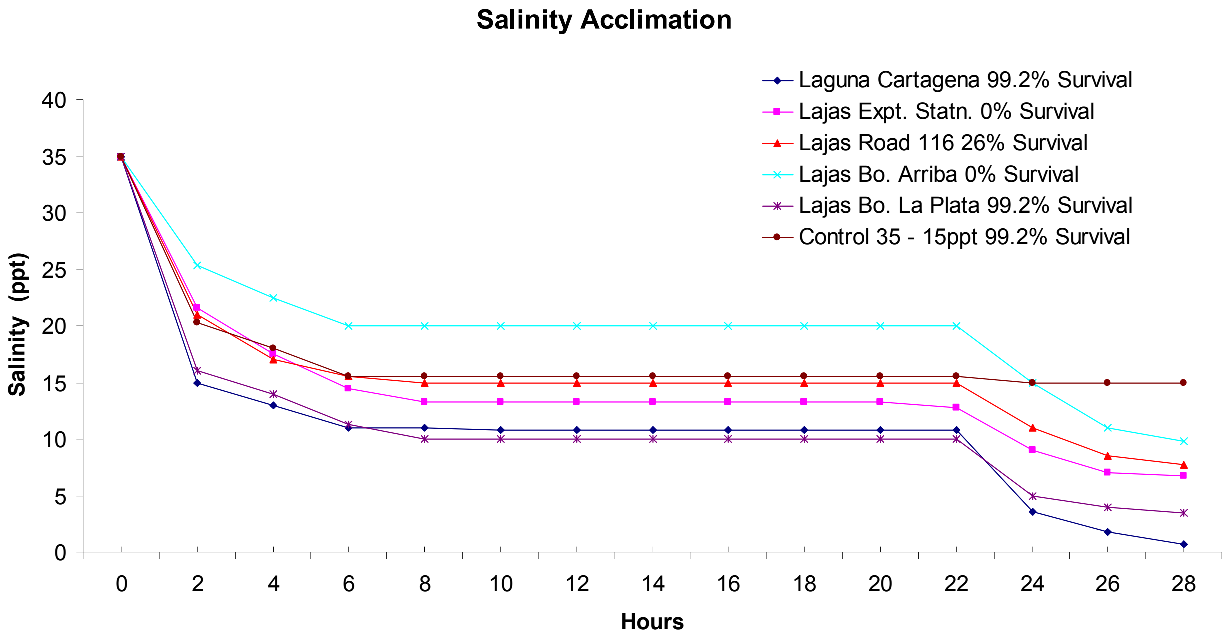


Figure 2.10 – Salinity measurements during low salinity acclimation.

The following survivals were recorded during the bioassay and are compared to estimated salinity of the waters to test the hypothesis that higher salinity would be more beneficial to the marine shrimp. This however, was not the case as can be seen from Table 2.2.

Table 2.2. Salinity vs. 48 h survival. *Salinities measured with a digital meter (+/-0.1 ppt) except for the control for which a refractometer was used (+/- 1 ppt). Three replicates each.

Source Water	Salinity (ppt)*	Average 48 h Survival (%)
Well Site 1	0.7	> 99
Well Site 2	6.7	0
Well Site 3	7.7	26
Well Site 4	9.8	0
Well Site 5	3.5	> 99
Control	15	> 99

Site # 1 and site # 5 were not significantly different from the control ($p \geq 0.05$), however sites # 2, # 3, and # 4 were significantly different ($p < 0.001$) based on a completely random ANOVA.

Table 2.3 AOV table for all treatments and for each experimental treatment vs. control.

AOV Table testing for differences between all treatments				
Source	SS	df	ms	F_{obs}
Between Samples	9586	5	1917.2	2300.6169
Within Samples	10	12	0.833342	
Totals	9596	17		
Conclusion: Since $F_{obs} > F_{tab}$, reject the null hypothesis of no differences between treatments				
AOV Table testing for difference between Site # 1 and Control				
Source	SS	df	ms	F_{obs}
Between Samples	0	1	0	0
Within Samples	1.33	4	0.33325	
Totals	1.3			
Conclusion: Since $F_{obs} < F_{tab}$, accept the null hypothesis of no differences between treatments				
AOV Table testing for difference between Site # 2 and Control				
Source	SS	df	ms	F_{obs}
Between Samples	3700.167	1	3700.2	22199.892
Within Samples	0.6667	4	0.166675	
Totals	3700.8337			
Conclusion: Since $F_{obs} > F_{tab}$, reject the null hypothesis of no differences between treatments				
AOV Table testing for difference between Site # 3 and Control				
Source	SS	df	ms	F_{obs}
Between Samples	2016.666	1	2016.7	930.7678
Within Samples	8.6667	4	2.16667	
Totals	2025.3267			
Conclusion: Since $F_{obs} > F_{tab}$, reject the null hypothesis of no differences between treatments				
AOV Table testing for difference between Site # 4 and Control				
Source	SS	df	ms	F_{obs}
Between Samples	3700.167	1	3700.2	22199.892
Within Samples	0.6667	4	0.166675	
Totals	3700.8337			
Conclusion: Since $F_{obs} > F_{tab}$, reject the null hypothesis of no differences between treatments				
AOV Table testing for difference between Site # 5 and Control				
Source	SS	df	ms	F_{obs}
Between Samples	0	1	0	0
Within Samples	1.33	4	0.33325	
Totals	1.3			
Conclusion: Since $F_{obs} < F_{tab}$, accept the null hypothesis of no differences between treatments				

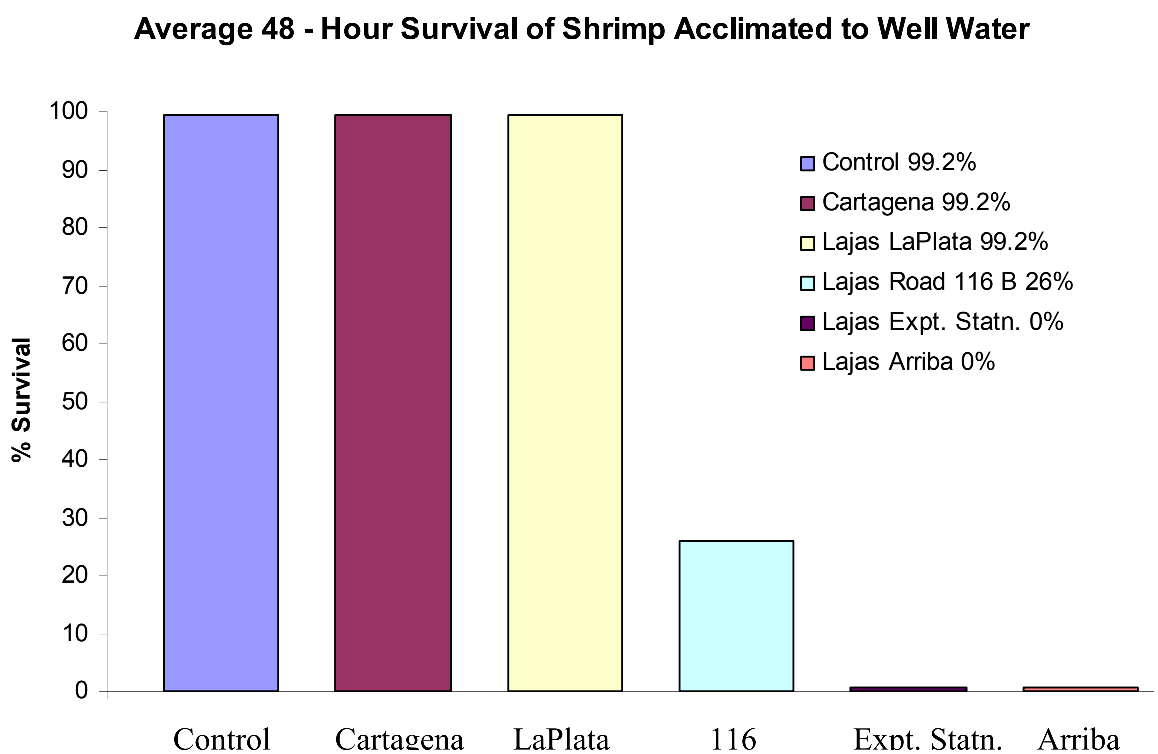


Figure 2.11 Average survival after 48 h low salinity acclimation, three replicates each.

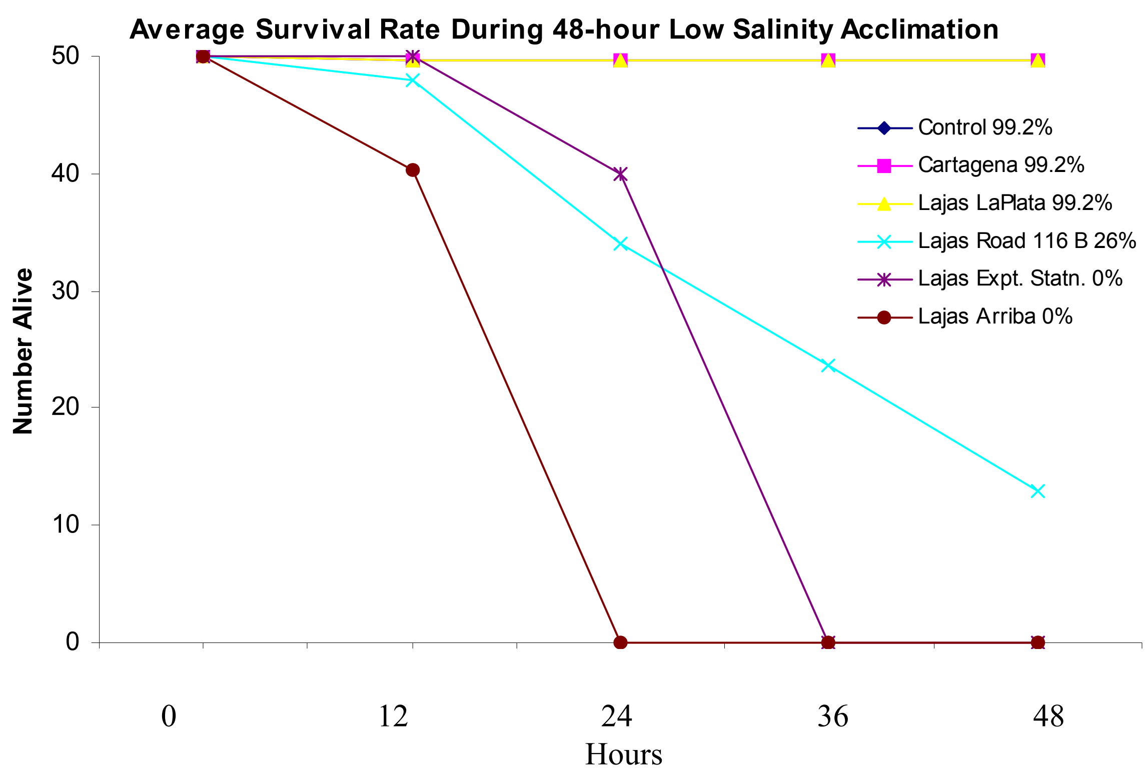


Fig 2.12 Average number of live shrimp during the 48 h low salinity acclimation.

2.5 Discussion

Comparing the results of the wells studied to those reported by Graves (1991) show ion concentrations have not changed over the last 19-21 years in sites 3, 4, and 5. No water quality data were reported by Graves (1991) for site # 1. Site # 2 ions have been concentrated. It is likely that water from the unconfined surface aquifer can enter the upper section of the well screen. Since the shallow aquifer is affected by evaporation during the dry season, the effect is concentration of the solutes and an effective increase in salinity. It is speculated that since well # 2 has a large section of the casing perforated, this allows water from different depths to mix within the well casing. Graves (1991) reported that ionic concentrations from Lajas Valley well water vary with depth even at the same site. Therefore, various methods (i.e. depth of well pump) of pumping could pull water from different depths resulting in different ionic concentrations. It is likely that differences in our ionic values are due to sampling differences, not a change in the aquifer. Well sites differ in many factors, so no single well water purging protocol is appropriate for all applications. Because well # 2 is a production well and not a piezometer, it is more difficult to completely purge the well casing. Nielson and Nielson (2006) mention the drawbacks of standard method well purging. They explain that piezometer wells are easier to purge completely as they are screened over a small depth range (usually < 1 m) and consequently are usually low production wells. Pumping causes significant drawdown, and effectively dewaters the well. New water from the aquifer enters the well casing and analysis will accurately reflect the aquifer's characteristics. Well # 2 does not draw down significantly during pumping as a 24.4 m section of the casing is perforated. Water rushes in during pumping causing the draw down to be negligible and, instead of replacing the casing water, mixes with it. It is likely that the water in well # 2 was not purged as it was in 1984 during construction.

Graves (1991) does not specify how these wells were purged in his report. However, during well construction, water is constantly pumped out as the auger digs. Therefore once the well is completely developed, sampling can be performed immediately, removing the effects of evaporation in the well casing. It is unlikely this well was pumped after its initial construction (1984) until this study in 2005. After 21 y, evaporation would cause ionic concentration in the casing water if turnover was negligible. Incomplete purging before analysis could also produce results such as ours. Based on this, it has been determined that ionic properties of the connate aquifer below the Lajas Valley have not changed and are stable over time (19-21 y).

When the relative concentrations of potassium (K) and sodium (Na) ions from the well samples are compared with known levels in standard seawater, it is apparent that ionic ratios are not consistent throughout the Lajas Valley's ground water. Libes (1992) reports that K is 3.6% Na in standard seawater. Diluted seawater has the same ionic ratios as full strength seawater (when diluted with distilled water). Based on this, it was hypothesized that potassium content was deficient for sites # 2, # 3, and # 4 which also had poor survival. Other researchers have experienced similar results (Boyd et al., 2006) who indicated that well waters in Alabama are deficient in K.

Of the five wells surveyed, # 1 and # 5 produced excellent survival (> 99%) and appear to be appropriate for low salinity shrimp acclimation with *L. vannamei*. Interestingly, these wells have the lowest total salinity of the five wells surveyed. Survival in water from sites 2, 3, and 4 (6.7, 7.7, and 9.8 ppt respectively) was 0, 26, and 0%. These results were contrary to the hypothesis that sites with higher salinity water would be more favorable for marine shrimp culture. In this case there is an inverse relationship between salinity and 48 h survival, which is contrary to what McGraw et al. (2002) reported. Endpoint salinity was not found to affect survival for PL's older than 15 days, when the endpoint salinity was between 1-12 ppt.

The acclimation procedure used demonstrates that PL_{18} can acclimate in 28 h to salinities as low as 1 ppt. This concurs with (McGraw et al., 2002) as their results showed that shrimp older than PL_{10} could be reduced to 1 ppt in 5-15 h without significant survival differences. Acclimation rate did not appear to affect survival during this trial. Due to the variable salinity of the target source water, the gradual addition of similar amounts of water from each well site produced different salinity reduction rates (Figure 2.10). Lajas Arriba (well site # 4, salinity 9.8 ppt) had the slowest reduction rate, and was closer to the target salinity end point sooner due to the proximity of the start and end salinities. The slowest total reduction rate, and the highest overall salinity yielded 100% mortality before 24 h had passed. This clearly indicates that the adjustment rate was not influencing survival during the low salinity acclimation.

2.6 Conclusions

Given the natural composition of ground water in the Lajas Valley, some sites appear adequate for shrimp acclimation to low salinity ground water, while others without modification are not. Salinity ranging from 0.7-3.5 ppt is better for this acclimation than salinities of 6.7-9.8 ppt. However, as discussed previously, salinity is not always the best indicator of adequate water quality for shrimp culture. It will be shown in chapter 3 that ionic ratios are more important than over all salinity for determining suitability of ground water for culture of *L. vannamei*.

From this experiment it can be concluded that the La Plata region near well site # 5 contains the best natural water quality for low salinity shrimp acclimation and is the most appropriate area for further studies of this nature due to the additional fact that much of this land is marginal for traditional agriculture due to saline soils. Construction of a demonstration facility in the marginal lands of this area is proposed. This area would be the most appropriate for further study based on the areas underutilized status and the saline characteristics of the soils. In addition, the central drainage canal is nearby which for aquaculture purposes is an important piece of existing infrastructure. The following figure (Figure 2.13 (NRCS 2008) is a soil map of marginal lands indicated by the blue box shaded region.

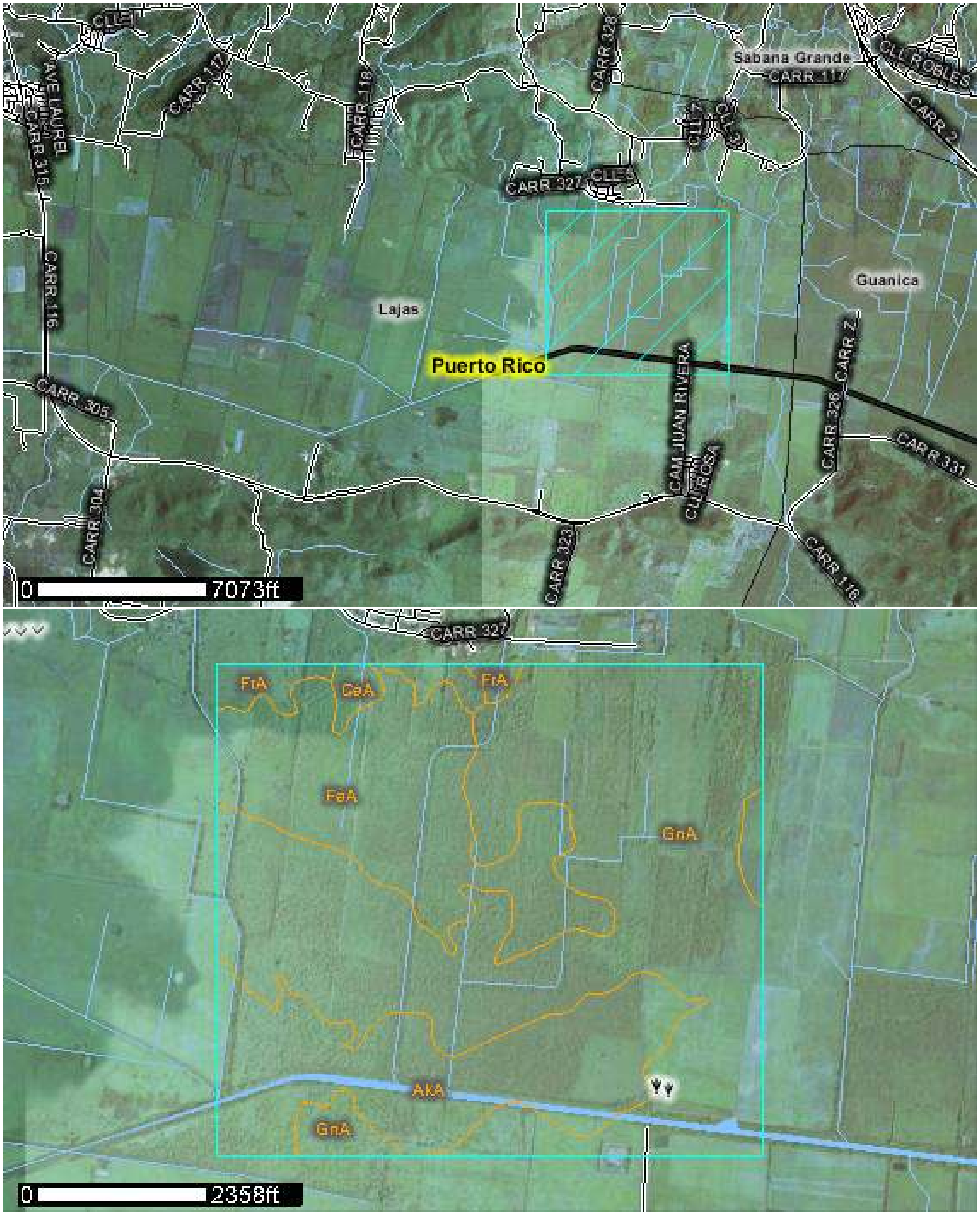


Figure 2.13 Soil types near a proposed future study site.

Soils types in Figure 2.13 are: 55.9% Guanica Clay, 20.8% Fe Clay, 19.7% Aguirre Clay**,** 1.9% Fraternidad Clay, and 1.7% Cartagena Clay**.** The total area of interest is

1174.2 acres. The boxed area indicates the best land area for potential development of a demonstration facility. Vegetation growing in that area is predominantly Mesquite (*Prosopis juliflora*) which is considered by the World Conservation Union as one of the world's most problematic invasive species with little commercial value. This species quickly invades abandoned pasture land in this area. In Puerto Rico, it is occasionally used for fence posts and charcoal.

It is apparent that some areas in the Lajas Valley have ground water that is appropriate for this type of shrimp culture. Ground water was toxic to *L. vannamei* in some cases presented here. In samples with poor survival, most shrimp began to show signs of stress within 12 h, and were dead before 36 h. By comparing the ionic concentrations of the major ions listed above to those of standard seawater, it was hypothesized that mortality was due to the *relatively* high Na to K ratios. Since potassium is found at 3.6% the concentration of sodium in standard seawater, the three wells with poor survival showed an enormous deficit in potassium in relation to sodium. The two wells with good survival had Na to K ratios closer to that of standard seawater, although for site # 5 K was still low. It is known that potassium plays a major role in shrimp physiology and in nerve function at the cellular level thus lending validity to the hypothesis that K deficiency was responsible for high mortalities in the higher salinity waters. With the addition of fertilizer containing the deficient ion (K), acceptable survival in other sites might be obtained. Additional experiments were designed to determine if potassium was the most important limiting ion in this case.

CHAPTER 3 - ACCLIMATION TO GROUND WATER WITH ADDED POTASSIUM

3.1 Balancing Ionic Ratios

Results from phase one of this investigation posed new questions. Mainly, why was the higher salinity water toxic to the saltwater shrimp when seawater is two to four times saltier than the waters tested? Salinity is a general description, and by definition includes all dissolved ions in solution. Apparently, salinity alone is not enough to use as an indicator for successful culture of marine shrimp.

It has been known for some time that one can not grow marine shrimp in water with NaCl, even if the salinity is 35 ppt (standard seawater). For instance, in Alabama, low salinity ground waters have been found to be deficient in potassium (K), and magnesium (Mg). Addition of potash and Kmag® (potassium magnesium sulfate) can be used to correct the problem (McNevin et al., 2004).

More recently, another experiment was conducted in order to evaluate the effects of several aqueous K and Mg concentrations. Roy et al. (2007) report on survival, growth, and respiration in juvenile *L. vannamei* reared in low salinity (4 ppt) well water in Alabama. Two experiments, a 14 d trial with post larvae and a 7 week trial with juvenile (0.2 g) shrimp were conducted to evaluate effects of K supplementation to culture water. Four different levels of K (5, 10, 20, and 40 mg/L) were utilized and a treatment of 4 ppt reconstituted seawater was used as a reference for comparison to ideal ionic ratios. Additionally, a 6 week growth trial using 1 g juvenile shrimp was performed to evaluate the effects of five concentrations of Mg (10, 20, 40, 80, 160 mg/L). Following completion of growth trials, measurements of basal respirometry rates were conducted to assess stress. Results from the 7 week K growth trial indicated significant differences ($P < 0.05$) in survival and growth among treatments. Individual weight, specific growth rate, and percent weight gain appeared to increase with increasing K concentration (decreasing Na:K ratios). Results from the Mg experiment revealed a significant difference in survival between the lowest Mg treatment (60%) and all other experimental treatments (90-97%). However, no differences in growth were observed. Shrimp respiration in the lowest Mg treatment (10 mg/L) was significantly higher than in the 80 mg/L) treatment. Roy et al. (2007) concluded that these results suggest a potentially higher energetic cost associated with depressed aqueous Mg concentrations that are common in low salinity environments. Cheng, et al. (2005) also stress the importance of Mg as they report on dietary Mg requirement and physiological responses of marine shrimp *L. vannamei* reared in low salinity water. An 8-week feeding experiment was conducted to determine the dietary magnesium (Mg) requirement and physiological responses of *L. vannamei* in low salinity water of 2 ppt. The dietary Mg requirement for optimal growth was 2.60-3.46 Mg/kg by using the polynomial regression analysis based on growth.

Comparing water quality results from chapter 2 with this data (Table 2.1), it can be seen that well water from the Lajas Valley is low in K but appears to have sufficient Mg.

3.2 Materials and Methods – K Ion Supplementation

Aquaria were set up in the same manner as in previous experiments. K_2SO_4 (sulfate of potash, 50% K) was purchased from Ochoa fertilizer company in Guanica, PR for a cost of $18.00 / 23 kg (50 lb) bag. Each water sample was tested with three replicates for statistical purposes. The control was untreated well water. Six tanks were filled with water from well site # 2, three of these were left untreated while the other three received a small amount of muriate of potash (K_2SO_4) see Table 3.1 for exact amount added. Since it is known that in standard seawater, K is 3.6% of Na, the deficiency of K in the well water was mathematically deduced. Additionally, another six tanks were filled with well water from site # 4, and as before, half of these aquaria were left untreated while the other three received the appropriate amount of K_2SO_4. Twelve empty tanks were placed directly below the treatment and control tanks. A small amount (7.6 L) of full strength (35 ppt) artificial seawater was placed in each of the 12 tanks. Fifty post larval shrimp (PL_{40}) were placed in each bottom tank and water was subsequently dripped in (~ 7.6 L/hr) over a period of 9 h. Once the aquariums containing shrimp had been filled, water was siphoned out as previously described to remove the artificial seawater. These tanks were then filled slowly over the next 9 h with well water. Survival was observed over 48 h. Live artemia was fed twice daily during the trial.

Following this, a second series of experiments were conducted using KCl in place of K_2SO_4 to compare results between two different K sources. KCl (51% K) is also available from Ochoa in 45 kg (100 lb) sacks for about $10.00. The acclimation procedure was identical to the previously mentioned protocol. Refer to Table 3.1 for exact amounts of KCl added.

Experimental design for these experiments was similar to the previous acclimation trial and a completely random ANOVA was used to determine statistical significance.

Table 3.1 Concentration of K and Na from well sites 2 and 4, and amount of K_2SO_4 and KCl added to 76 L.

Well site	**Overall Salinity**	**Na (mg/L) Reported**	**K (mg/L) Reported**	**K deficient mg/L**	**Compound added**	**% K in compound**	**Total added (g)**
2	6.7 ppt	1800	3.5	61.5	K_2SO_4	50%	9.4
4	9.8 ppt	3300	40.3	114.5	K_2SO_4	50%	17.4
2	6.7 ppt	1800	3.5	62.08	KCl	51%	9.04
4	9.8 ppt	3300	40.3	114.91	KCl	51%	16.7

3.3 Results

Survival was recorded over a 48 h period and was the following (Table 3.2 and Figure 3.1). Addition of K_2SO_4 and KCl to water completely ameliorated 48 h mortality, producing > 99% survival after 48 h.

Table 3.2. Survival in treated vs. untreated well water.

Control well # 2	48 h survival 18%
K_2SO_4 added # 2	48 h survival > 99%
KCl added # 2	48 h survival > 99%
Control well # 4	48 h survival 0%
K_2SO_4 added # 4	48 h survival > 99%
KCl added # 4	48 h survival > 99%

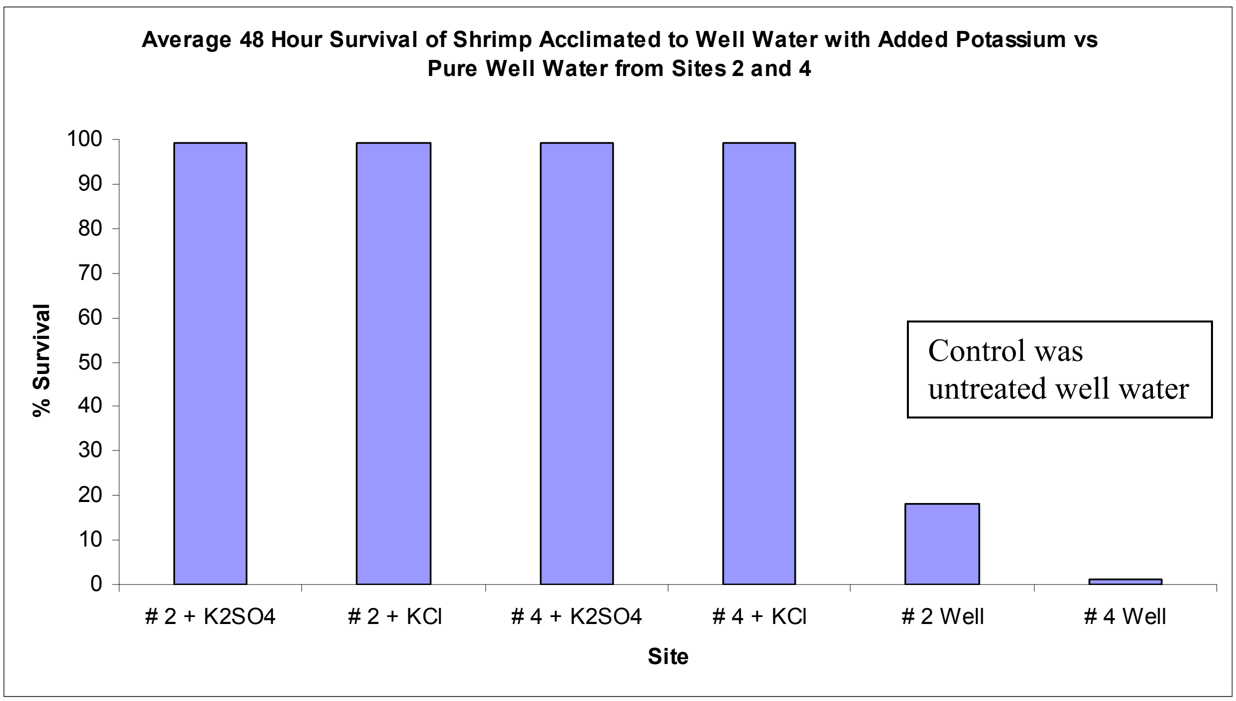


Figure 3.1. Average 48-h survival in water with and without added K, 3 replicates each.

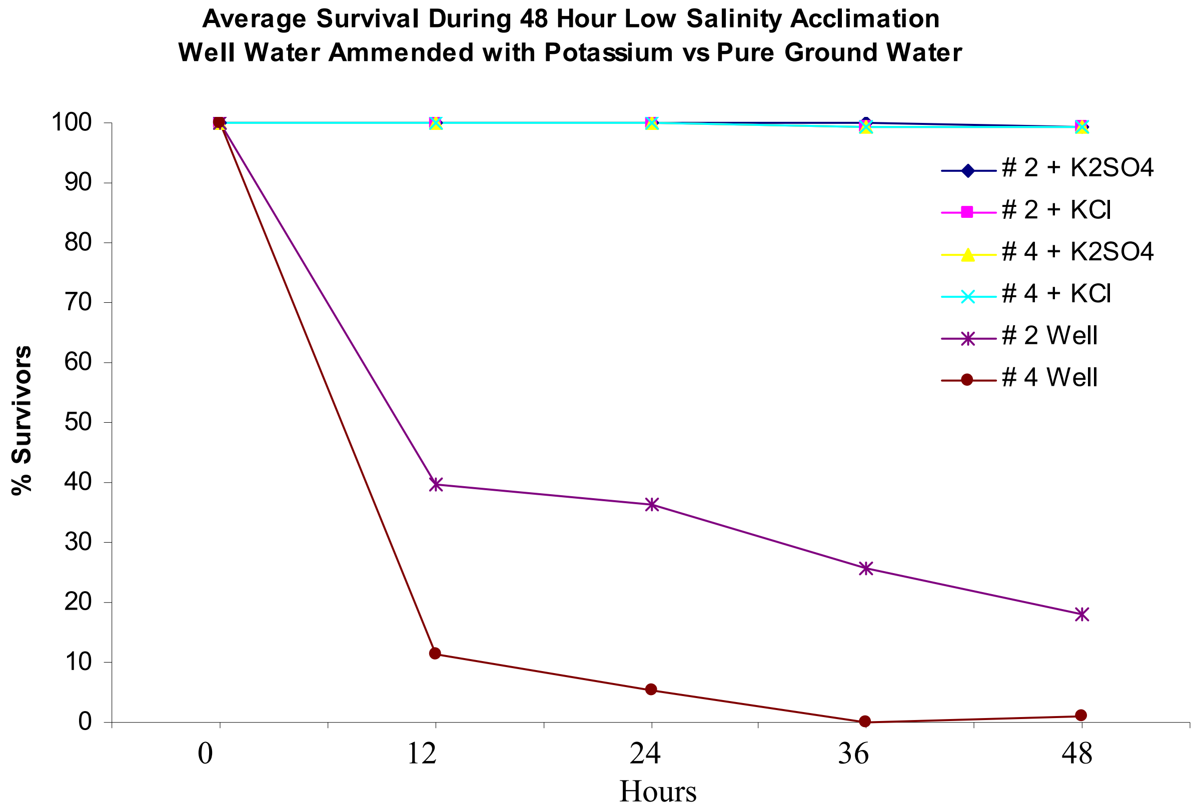


Figure 3.2. Average survival during acclimation to water with and without added K, three replicates each.

Results from this trial were highly statistically significant ($p < 0.001$) when the completely random ANOVA statistics were calculated. Trials with added K had much greater survival than the control which was pure well water.

Table 3.3 AOV table for treatments of added K vs. pure well water.

AOV Table testing for differences in treatments: added potassium vs. pure well water				
Source	SS	df	ms	F_{obs}
Between Samples	4933.66	2	2466.7778	11100.776
Within Samples	1.333	6	0.2222167	
Totals	4934.8889	8		
Conclusion: Since $F_{obs} > F_{tab}$, reject the null hypothesis of no differences between treatments				

3.4 Discussion and Conclusions

K appears to be the most important limited ion present in ground water from these sites. Increasing K to 3.6% of Na resolved mortality problems over a 48 h period. This correlates with other researchers as they have also discovered positive relation between K levels and survival during acclimation to low salinity. Davis et al. (2005) raised both K and Mg to levels similar to what would be found in seawater diluted to 4 ppt. No significant differences in growth were obtained between treatments (i.e. K vs. Mg), but in both cases, adding ions increased survival significantly over the control (which was pure low salinity well water at 4 ppt). In addition, Davis et al., (2005) reported that when both survival and biomass (growth) were taken into account after a 21 d grow out, the results showed a positive K influence. This confirmed that similar short term bioassays were applicable in estimating longer term results.

These findings indicate that the Pacific white shrimp *L. vannamei* can indeed be acclimated successfully to ground water from the Lajas Valley. In addition, water from varied well depths and locations was utilized with only slight additions of a relatively inexpensive and common fertilizer containing K.

Marginally productive or underutilized areas of the Lajas Valley agricultural reserve would be the most likely sites for a larger scale grow out trial. Areas like the La Plata region are very appropriate for this type of study as they are currently the most underutilized areas in part due to prevalence of saline Guanica clay soils. The ground water in this area is also less brackish, hence requiring less K to balance the Na levels. Moreover, the threat of low salinity water contaminating the already saline soils is diminished.

Following this experiment, shrimp were left in aquaria for several weeks with light feeding of artemia and a larval feed (45% protein) made by Rangen. Variable survival was observed. Mortality was attributed to cannibalism on recently molted shrimp as feeding was kept to a minimum to avoid water quality issues in the small aquaria. Data from the trial were inconsistent and highly variable due to the obvious problems associated with culturing shrimp in small aquariums for extended periods of time. However, it was concluded that in general shrimp can acclimate to well water throughout the Lajas Valley and under the correct management of water quality should be able to survive and grow normally. A larger scale recirculating tank was constructed to test this hypothesis.

CHAPTER 4 - LONG TERM GROWTH AND SURVIVAL

4.1 Introduction

Based on the results from short-term bioassays, it was concluded that *L. vannamei* can be successfully acclimated to Lajas Valley ground water. Since water quality through out the Lajas Valley is not homogenous, it was also determined that the suitability of ground water in some areas is improved dramatically by the addition of potassium rich fertilizer. The next step was to verify long-term survival and growth because the ability to grow to harvest size is the most basic requirement of shrimp aquaculture. One important study that evaluated the interaction of salinity and Na/K ratio in seawater on growth, nutrient retention and food conversion of juvenile *L. vannamei* is Zhu et al. (2006a). The effects of salinity, Na/K ratio and their interaction on growth, molting, nutrient retention and food conversion of *L. vannamei* were investigated. The test shrimp were reared in artificial seawater and fed fresh polychaete worms, *Neanthes japonica* (Izuka) for 30 d. Two salinities (30 and 15 ppt) and 5 Na/K ratios (25.6, 34.1, 47.3, 102.1 and 153.3, mmol/mmol) were set, and each treatment had four replicates. During the feeding trial, the molting frequency was affected by neither salinity nor interaction effects, but Na/K ratio had an effect ($P < 0.05$). And there was a tendency that molting frequency rose with the increment of Na/K ratio at both salinities. Final body weight, weight gain and specific growth rate were significantly influenced by salinity, Na/K ratio and interaction effects ($P < 0.05$), whose mean values at salinity 15 ppt were significantly higher than those at 30 ppt. At 30 ppt, weight gain and specific growth rate of shrimp exposed to Na/K ratio 34.1 and 47.3 were respectively more than 26 and 15% higher than of those exposed to the other ratios ($P < 0.05$), but no significant differences in growth were found among treatments at 15 ppt ($P > 0.05$). Na/K ratio and interaction effects significantly affected the nutrient retention, food conversion and protein utility of the shrimp ($P < 0.05$), whereas salinity showed little impact. At 30 ppt, the nutrient retention of shrimp exposed to Na/K ratio 153.3 were more than 30% lower than of those exposed to the other 4 ratios ($P < 0.05$); the food conversion of shrimp exposed to Na/K ratio 34.1 and 47.3 were more than 20% higher than of those exposed to Na/K ratio 25.6 and 102.1 ($P < 0.05$), and the food conversion of shrimp exposed to Na/K ratio 153.3 was even lower ($P < 0.05$). At 15 ppt, no significant differences in food conversion among treatments were found ($P > 0.05$). Protein utility showed the similar tendency as food conversion at both salinities. Most importantly, Zhu et al. (2006a) concluded that that shrimp were more adaptable to abnormal Na/K ratios at low salinity than at higher salinities, and good growth could always be obtained within a Na/K range of 34.1-47.3 (mmol/mmol) regardless of salinity.

4.2 Materials and Methods

A recirculating grow out system (1154 L) was constructed and prepared by the following method. 141 grams of K_2SO_4 was added to 1154 L of well water from site # 2. During the first week of November (2005), nursed PL's were acclimated slowly to and stocked at a density of 300 / tank (0.32 shrimp / L). Shrimp were fed to satiation daily with Tender Mills brand sinking shrimp pellet 30% protein. Aeration was constant in the system to keep dissolved oxygen saturated at all times. A previously conditioned biofilter kept general water quality (NH_4 and NO_2) levels within healthy ranges for shrimp culture.

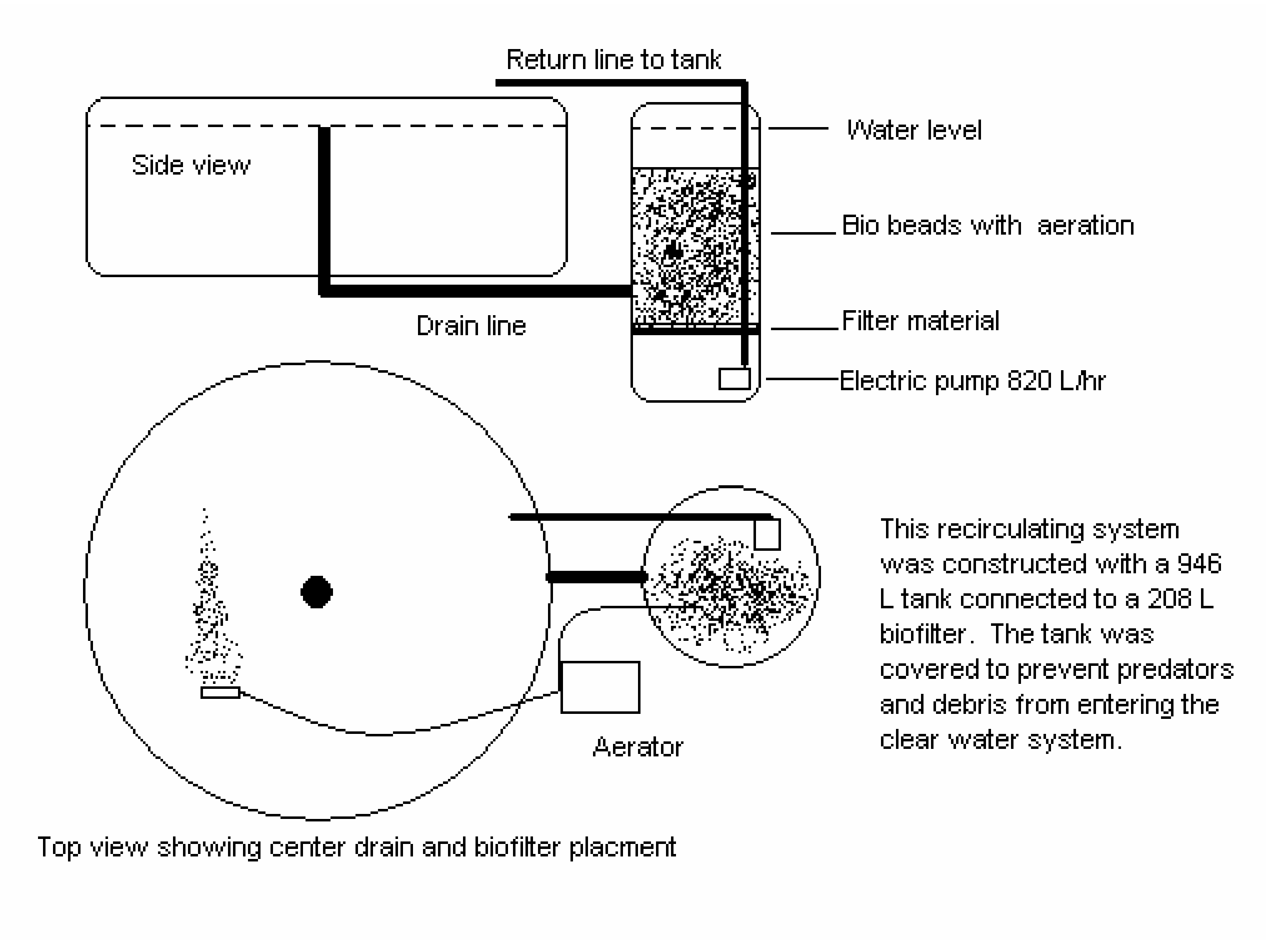


Figure 4.1. Schematic diagram of the recirculating system used for the grow out study.

4.3 Results

Survival was calculated by counting dead individuals, and as the experiment matured, by counting live shrimp. After two weeks, no mortality of shrimp stocked was observed. On November 24th, 2005 (3 weeks post stocking) one individual was observed being cannibalized. This individual was a soft shell, and had recently molted. Regular feeding helped to reduce this problem, but never solved it. When fed pellets, shrimp released cannibalized individuals and switched to the artificial diet. Cannibalism continued throughout the experiment resulting in the death of recently molted individuals. One or two shrimp were observed being eaten every few days. Estimates of 165 individuals present on May 7th yield a 6-month survival rate of 55%.

Growth rates were relatively slow over the cold months (Figure 4.2). On May 7th, 2006 a sub-sample was taken and shrimp were averaging 7 g.

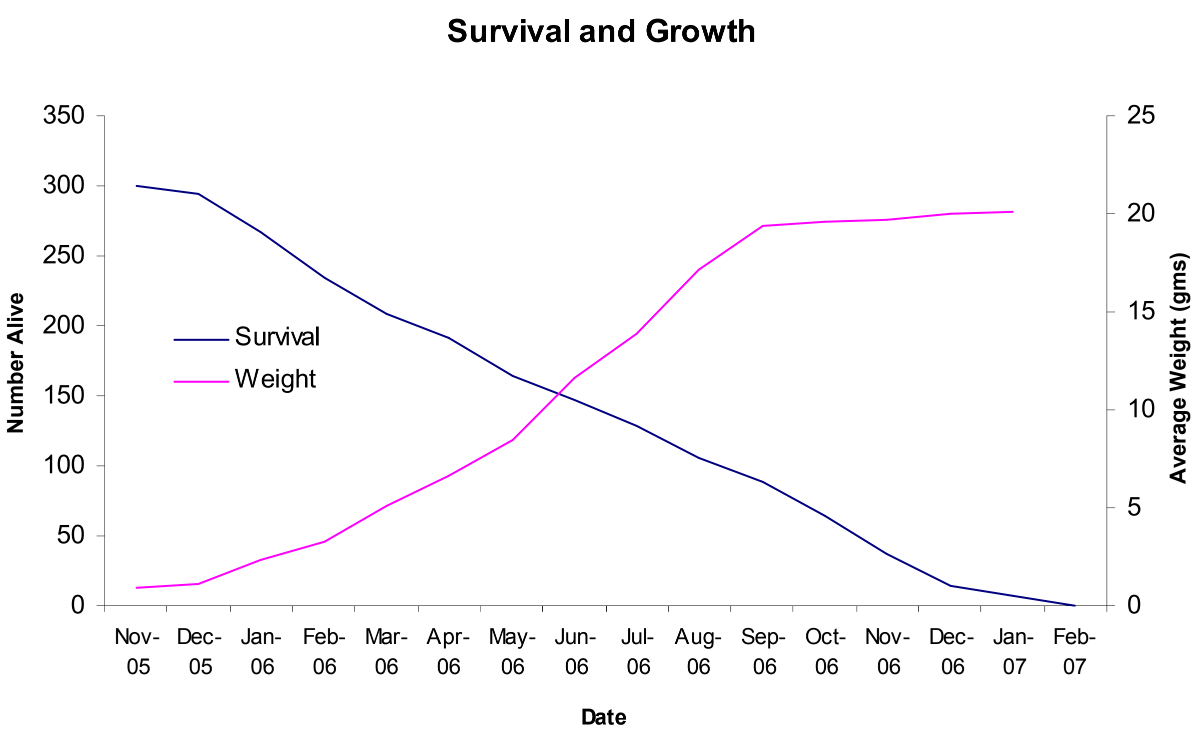


Figure 4.2. Survival and growth over 1.25 y in a recirculating system.

4.4 Discussion

Typically, in tropical countries, grow out times range from 4 to 6 months. During this trial, survival at 6 months was estimated at 55%. Mortality was due to cannibalism as dead shrimp were found being eaten or exhibiting evidence of cannibalism. All shrimp found dead were soft shells and the molts were always evident in the drain collection area of the tank. This leads to the hypothesis that the feed used might not have been sufficient to satisfy the nutritional needs of grow out *L. vannamei* under these conditions. Shrimp were fed once daily with a sinking shrimp pellet (30% protein, Tender Mills Feed). There is little question that a quality feed for aquaculture purposes is important and fortunately, there is considerable research available from the literature on what feed is best for culturing the marine shrimp *L. vannamei*. Based data from a similar experiment (Gonzalez-Felix et al., 2007), this protein content is appropriate for grow out of *L. vannamei* raised in a low salinity zero-water exchange culture system. They cultured juvenile *L. vannamei* over 4-weeks to evaluate the effects of different dietary protein levels (25, 30, 35 and 40%) on the growth and survival of shrimp raised in a low salinity (4.6 ppt), zero-water exchange culture system, as well as on the nitrogen budget and ammonia efflux rate. No significant differences were observed among the dietary treatments for final weight, weight gain or survival of shrimp, although the best performance was observed in the 25% protein treatment group as less nitrogen was lost to the system as NH_4. However, they concluded that the 25% protein diet was also better under these conditions, presumably, because it provided more carbon for heterotrophic bacteria (which shrimp can eat) and reduced the nitrogen loading of the system. Since the recirculating system used for our trials was clear water (i.e. little or no heterotrophic bacteria in the culture tank), it is hypothesized that the 30% protein feed used may indeed be lacking some of the dietary requirements for marine shrimp when utilized as the sole food source in a closed system over extended periods of time. This was the only shrimp feed available locally, and may not contain sufficient protein required for *L. vannamei* grow out animals of this size in clear water systems. For adult shrimp, Harbor Branch recommends a quality feed of no less than 35% protein (Van Wyk et al., 1999). Other researchers concur. Sowers et al. (2006b) used a 35% protein feed with 2.5% squid added during their low salinity production trials.

In clear water recirculating systems, shrimp are 100% dependent on the feed for nutrition (discounting cannibalism). In Hawaii, researchers have increased growth rates, and reduced food conversion ratios by utilizing 'green water' recirculating systems (Izquierdo et al., 2006). They suggested that one of the causes for the better performance of *L. vannamei* reared in green water in comparison with clear water is the shrimp's ability to obtain additional nutrients from food organisms endogenously produced within the green water system. However, the nutritional components responsible for these effects had yet to be determined. They focus on understanding the importance of natural food organisms in zero-water exchange systems as source of essential fatty acids for the *L. vannamei*. Five treatments were tested: two conducted in mesocosm systems (green water) with shrimp-fed diets containing either fish oil or olive oil, and another three conducted in clear water with shrimp-fed diets containing either olive oil, a docosahexaenoic acid rich oil or an arachidonic acid rich oil. Their research led them to conclude that in clear water, it is necessary to include at least 4.8 g/kg docosahexaenoic acid in diet dry weight. In green water systems, the nutritional contribution of the floc to shrimp in the mesocosm culture reduces or eliminates the need for a dietary source of fish oil and illustrates the importance of docosahexaenoic acid and arachidonic acid to enhance shrimp survival in clear water conditions. In addition, this species has been shown capable of converting bacterial protein into useful nutrition in traditional earthen pond culture, where shrimp gather much of their nutrients from natural productivity (Fox et al., 2001).

One of the most promising areas of research for this species is in incorporating a bacterial based floc to increase the natural productivity component of the shrimp's diet. Wasielesky et al. (2006) has reported on the advantageous of a floc based culture system. They specify potential benefits of the effects of natural production in a zero exchange suspended microbial floc based super-intensive culture system for *L. vannamei*. Zero water exchange, super-intensive culture of shrimp in enclosed raceway type systems can be considered environmentally friendly in that containment of water within the system prevents potential spread of disease between the wild populations and cultured animals and avoids nutrient rich waste from polluting coastal waters. However, as a relatively new strategy for shrimp production, there is much still to be learned about the potential biological and economic benefits of producing shrimp in suspended microbial floc based systems. Understanding shrimp feeding behavior and quantification of shrimp feed consumption provides valuable information for culturists to improve feed management, one of the keys to economic viability. The objective of their study was to evaluate the nutritional contribution of varying levels of microalgae/bacterial floc on survival, growth, food consumption, and food conversion ratio of *L. vannamei* juveniles fed diets with different protein levels in replicated experimental microcosm tanks. The 20 d experiment evaluated 9 treatments, three water types fed three different protein diets. Water was recirculated within a sump and consisted of either clear, UV filtered water, water containing microbial floc from an adjacent zero exchange super-intensive raceway production unit, or a 50:50 mix of clear water and raceway water. Diet treatments were either no food, 25 or 35% protein content. Treatments were randomly assigned to 50 L, mesh covered plastic bins receiving each water type. Each treatment consisted of five replicates, each containing 44 shrimp, with a mean stocking weight of 1.82 +/-0.71 g for a final density of 300 per m^2. Shrimp in each treatment (except the no feed treatment) were fed 3 times daily via a specially designed feed tray. Food consumption and food conversion ratio were calculated based on weight gain, survival, total consumed feed, feed loss through leaching, and initial feed moisture content. Results were analyzed by two-way analysis of variance (ANOVA) and differences between the means analyzed by Tukey's Test (alpha = 0.05). Survival in the fed treatments was greater than 98% in all treatments ($P > 0.05$). Survival in the non-fed treatments was significantly higher in the raceway water treatments than in the clear water treatment ($P < 0.05$). Final weight, weight gain, final biomass, food consumption and FCR were significantly higher ($P < 0.05$) in all treatments fed with 35% protein feed. This result suggests a positive relationship between the growth parameters and the protein content of the feeds in this system, and confirms the benefit of natural productivity for production of *L. vannamei*.

Other reports have shown that in ponds, the protein source used to formulate the feed can be changed without significant changes in growth or survival. Amaya et al. (2007a and 2007b) have shown that fishmeal can be completely removed from feeds for *L. vannamei* grown in ponds. This study (Amaya et al., 2007a) evaluated a fish meal replacement strategy using vegetable protein sources in practical feeds for marine shrimp reared in ponds. Juvenile *L. vannamei* (0.03 g) were stocked into sixteen 0.1 ha low-water exchange ponds and reared over an 18-week period. Four commercially extruded diets formulated to contain 35% crude protein and 8% lipids were evaluated. These diets included varying levels of fish meal (9, 6, 3, and 0%) which was replaced by a combination of increasing levels of soybean meal (32.5, 34.9, 37.2 and 39.6% respectively) and corn gluten meal (0.0, 1.7, 3.2, and 4.8% respectively) to replace the protein originating from fish meal. At the conclusion of the experimental period, there were no significant differences ($P \geq 0.05$) in shrimp production among the test diets. Mean final yield, final weight, feed conversion ratio and survival values ranged from 5363-6548 kg/ha, 18.4-20.7 g, 1.38-1.12 and 84.0-94.0%, respectively (Amaya et al., 2007a). These same researchers (Amaya et al., 2007b) also report that fish meal can be replaced with plant protein sources in diets including 16% poultry by-product meal without affecting shrimp growth and production in an outdoor tanks system.

Some research has indicated that for low salinity culture of this species, adding dietary supplements to feeds has lead to improved results. One such supplement is astaxanthin which is a powerful antioxidant carotenoid. Like many carotenoids, it is a colorful, fat/oil-soluble pigment. Astaxanthin can be found in microalgae, yeast, salmon, trout, krill, shrimp, crayfish, crustaceans, and the feathers of some birds. Currently it can be used by both animals and humans, approved by the FDA as a food coloring. Flores et al. (2007) report the effects of dietary astaxanthin supplemented at 0, 40, 80 or 150 mg astaxanthin/kg on growth, survival, molt frequency, osmoregulatory capacity and selected metabolic and haematological variables in *L. vannamei* acclimated to low-salinity water (3 ppt). When astaxanthin was supplemented at 80 mg/kg, improved growth, survival and molt frequency were observed. The lowest osmoregulatory capacity was also exhibited in shrimp fed with dietary astaxanthin at 80 mg/kg. Shrimp haemolymph concentrations of glucose, lactate, haemocyanin and total haemocyte count were all significantly enhanced by feeding the diet supplemented with 80 mg/kg astaxanthin compared with shrimp fed with the other diets. Flores et al. (2007) concluded on the basis of their results, a dietary astaxanthin supplementation of 80 mg/kg was best for juvenile *L. vannamei* cultured in low-salinity water.

Unfortunately, feeds can not replace the need for proper ionic balance in the culture system. Although in some cases, marginal benefits of quality feeds have been shown (Saoud et al., 2007) the same authors also specify that dietary supplements have been proposed as a potential remediation strategy to counteract mineral deficiencies in low-salinity well waters used for shrimp culture in Alabama have not been successful. Results from their study indicated that dietary supplementation of K (1.0%) in the absence of an appropriate ionic profile failed to enhance growth or survival of *L. vannamei*.

Saoud and Davis (2005) report on the effects of Betaine supplementation to feeds of *L. vannamei* reared at extreme salinities. They note that as shrimp production worldwide has increased dramatically, optimal sites are no longer abundant. Consequently new farms are being constructed in areas where water salinity and ion composition are suboptimal. Aquaculturists and feed suppliers are attempting to alleviate ion nonequilibriums through nutrition. One nutritive supplement that has been marketed is the amino acid betaine. The present work evaluated the effects of betaine as a feed supplement on the survival and growth of *L. vannamei* reared at extreme salinities (0.5 or 50 ppt). Survival (75-89%) and final weights (2.8-3.5 g) were typical for this species reared in indoor systems, but there was no significant influence of the presence of betaine. However, there was a significant influence of salinity on growth. These results suggest that betaine supplementation to practical diets designed for *L. vannamei* does not improve production at extremely low or high salinities.

In addition to this, other research has discovered that the incorporation of deficient ions in feeds can not make up for deficient ions in the low salinity well water. Zhu et al. (2006b) studied the effects of seawater potassium concentration on the dietary K requirement of *L. vannamei*. Effects were studied under the condition of artificial seawater at salinity 30 ppt for 56

d. Growth, feeding, nutrient retention and feed conversion efficiency of the shrimp were significantly affected by the K concentration in the seawater ($P < 0.05$), but the dietary K levels showed little effect. This indicated that *L. vannamei* may have poor ability of assimilating K from dietary sources efficiently at salinity 30 ppt, and dietary supplementation of K had limited effect on improving growth of the shrimp while environmental K was sufficient.

Also along these same principals, Roy et al. (2006) examined the effects of lecithin and cholesterol supplementation to practical diets for *L. vannamei* reared in low salinity (4 ppt) waters. They report that it has been suggested that increasing phospholipids (lecithin) and cholesterol in excess of dietary requirement improve osmoregulatory capacity in *L. vannamei*, thus leading to better survival and growth under low salinity conditions. Cholesterol is an essential sterol involved in the molting process in shrimp. Phospholipids are important in cholesterol transport, facilitate the storage of lipids in the hepatopancreas, an important energy reserve during the molting process and are an important component of cell membranes. In order to investigate the possibility of improving growth and survival under stressful (i.e. low K and Mg) rearing conditions, a series of lab and on-farm experiments were conducted. Their results indicate that the shrimp were stressed in both experiments, and there were no apparent benefits to supplementing lecithin and cholesterol in excess of the dietary requirement. Two on farm trials were conducted in parallel using either a mediated water source (Farm 1) to produce low stress waters. No benefits from lecithin and cholesterol supplementation in excess of the dietary requirement were observed when compared to the basal diet under any test conditions. Based on results of their present study, dietary supplementation of cholesterol and phospholipids in excess of the requirement is not warranted for *L. vannamei* reared in low salinity waters.

In our case, growth is difficult to compare with other research due to differences in stocking size and culture conditions. However, the fact that shrimp were able to reach harvest size under less than optimum conditions and survived for more than a year is strong evidence towards the potential for low salinity culture of this species in the Lajas Valley.

4.5 Conclusions

Results indicate that the marine shrimp *L. vannamei* can be successfully grown to harvest size in well water from the Lajas Valley. Some areas do not naturally contain sufficient K levels for normal survival. However, with the addition of inexpensive fertilizer available locally, this problem can be overcome. More research is needed to determine economic viability and sustainability at the commercial scale. Larger raceway or pond systems need to be evaluated for long term survival and growth, and to justify the added costs of addition of potassium fertilizer. A higher quality feed with 35% protein is currently (2008) available locally. This feed should be incorporated into future studies of this nature to see if cannibalism can be reduced. This would aid in demonstrating the true potential and economic viability for the culture of this marine shrimp in low salinity waters of the Lajas Valley.

A demonstration facility constructed in Barrio La Plata would be highly beneficial as it would demonstrate the potential for developing an inland low salinity shrimp culture industry in the Lajas Valley. A model for this follows (Figure 4.3).

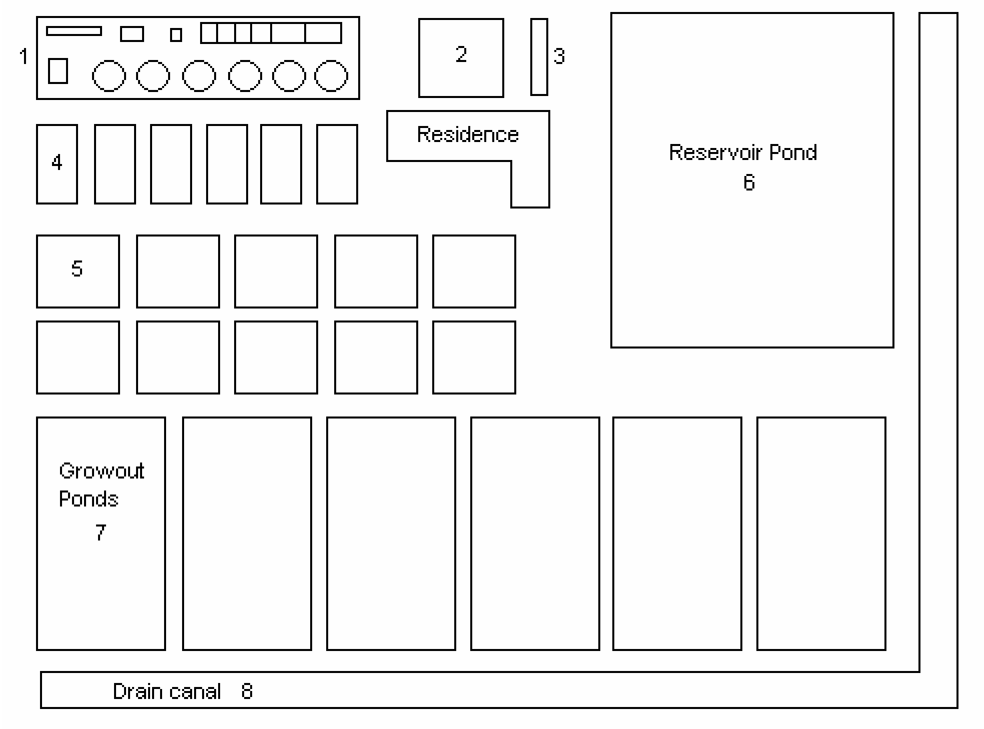


Figure 4.3. Model of a low salinity shrimp culture demonstration facility.

Legend: The acclimation hatchery building (1) should contain circular recirculating tanks for holding and acclimation of post larval shrimp. Also this building should house a system capable of temporary holding of live shrimp before processing, and necessary equipment to process and maintain processed shrimp before sale. This hatchery structure should be dome roof, with a shaded plastic green house cover. A storage building (2) for maintenance equipment such as tractors and harvest seines should be large enough to store heavy machinery and securable against storms and thievery. Aluminum-roofed hanger structures are most suitable for this. A feed storage area (3) can be a trailer conversion such as a Sea Land cargo container. They are metal and can be secured against nuisance animals such as rats and mice. Nursery ponds (4) are small ponds, 0.1 acre or less, used for the production of nursed post larvae. Medium sized grow out ponds (5) are useful for experimenting with high density, high energy input shrimp culture. Some research with this species has shown that very high density culture is possible when heavy aeration is applied to ponds and raceways which switch the pond microbial community from algae dominated to bacterial flocculent dominated. This flocculent is edible to shrimp, and when properly managed, excellent results can be achieved. These ponds should be about 0.25 acres in size in order for the water quality to be managed efficiently. A large reservoir pond (6) is located at a higher elevation than the remaining hatchery and ponds so that water from this pond can flow by gravity to all areas of the facility. A nearby well will supply ground water to this pond via electric, diesel, or wind generated pump. This pond should be at least 2 acres in size. Grow out ponds (7) can range in size from 0.5-2 acres, with a good average size being 1 acre. Ponds larger than this require substantial water and land use and are not practical for this type of shrimp culture operation. Ideally all ponds should be lined with plastic or rubber liners such as Yunker Plastics Inc. offers http://www.yunkerplastics.com/aquaculture.htm. All drains should lead to the drain canal (8) where water can be treated (settling pond details not shown) and recycled back to the reservoir pond. Finally a residence or office building should be centrally located with unobstructed views of the entire facility. This can be living quarters for the site manager or a support building and office for round the clock employees. Shrimp farms routinely require a 24 h presence to manage water quality in production ponds and deter unlawful trespassing. The facility should be well fenced around the perimeter. It will need power lines, communication (phone/fax), and potable fresh water. A backup diesel power generator would also be necessary to provide uninterrupted power to the facility in the event of a power outage. A reasonable access road to the facility is also a basic requirement.

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Appendix

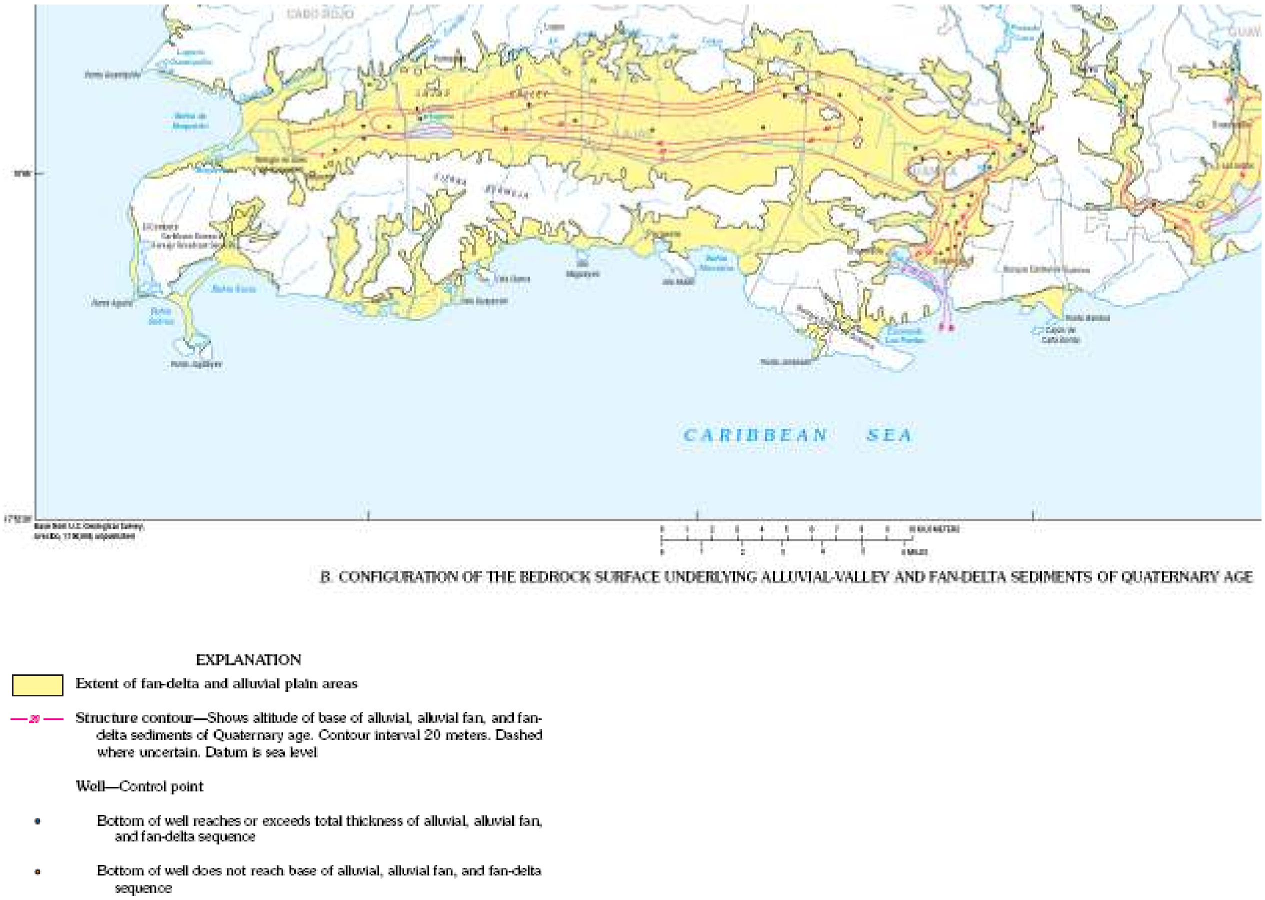

Figure A1. Configuration of bedrock surface underlying alluvial sediments in Lajas. Reprinted from (Renken et al., 2002).

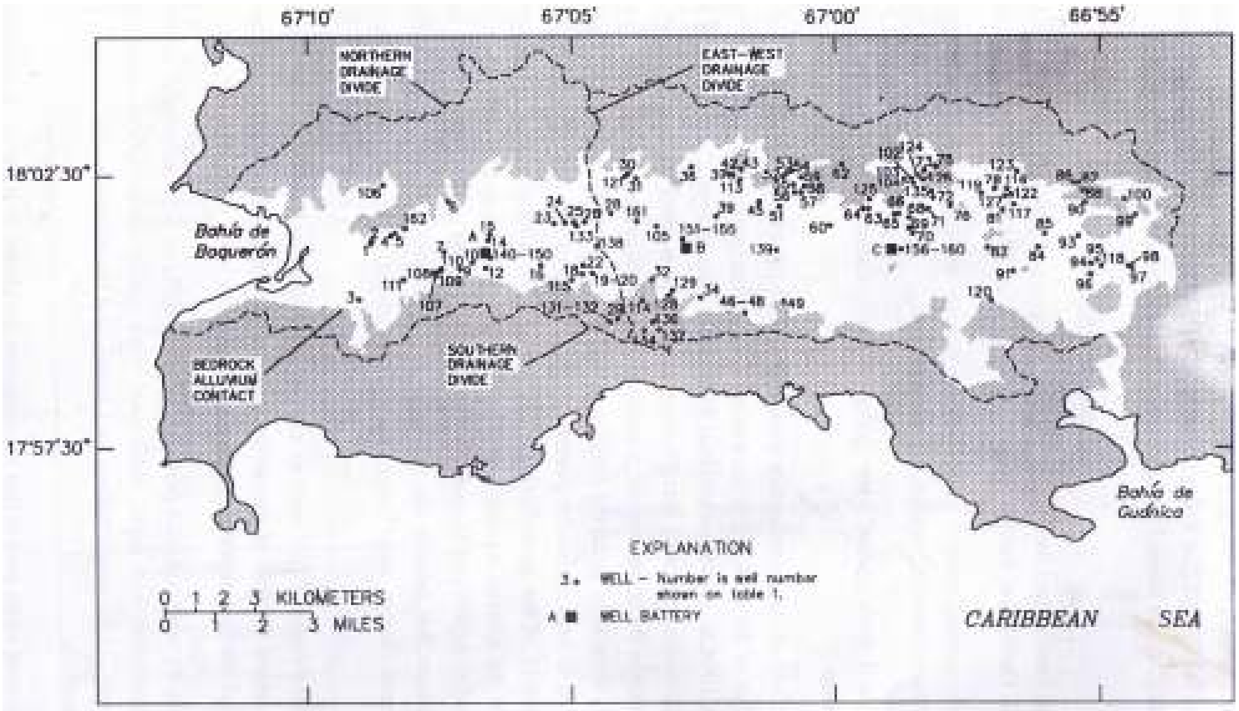

Figure A2. Location of all well sites surveyed during 1983-1986. Reprinted from Graves (1991).

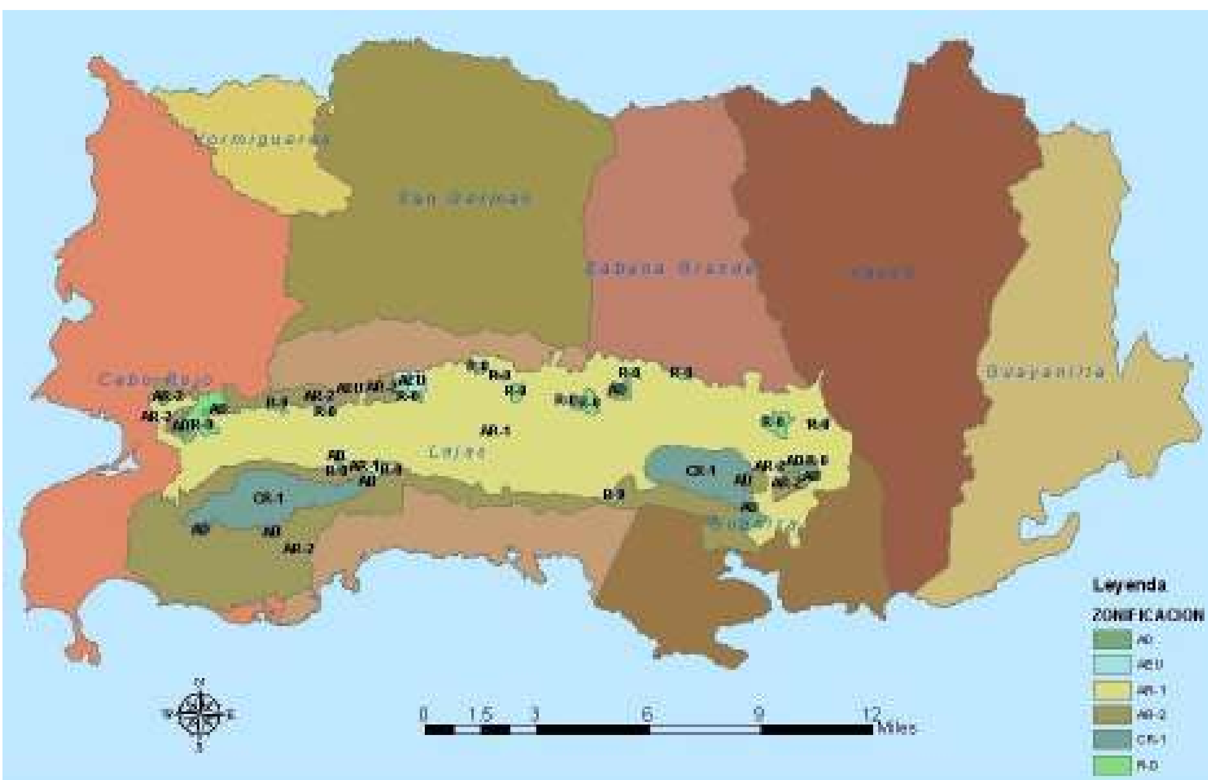

Figure A3. Map of the Lajas Valley Agriculture Reserve. Puerto Rico Planning Board (2007).