Knowledge, perceptions, and practices with respect to the prevention of dengue in a mid-Pacific coastal village of Costa Rica

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Abstract: Dengue fever is the fastest spreading, most prevalent and deadly arthropod-borne viral disease worldwide, present in over 125 countries. In 2013, Costa Rica is experiencing the highest number of Dengue infections since the virus’s reintroduction to the country in 1993. This study evaluated the Dengue-related knowledge of 320 community members, and polled opinions on how to better educate and mobilize the community on Dengue prevention in Quebrada Ganado, Costa Rica during March-May, 2013. In addition, property inspections were used to find relationships between knowledge or opinions and presence of Aedes breeding sites. Results showed that while citizens knew the virus is transmitted by mosquitoes that reproduce near water, they lacked knowledge on Aedes habits and confused it with other mosquitoes. Eighty-one percent of respondents assumed some responsibility for dengue prevention. Suggestions for improved education included consistent and continual information on the risks and dangers dengue poses to an individual. Characteristics relating to households with more positive breeding sites were: lower education level, higher prevalence of dengue infections in the household, lesser knowledge of dengue symptoms, a lower rating on the dangers of dengue and a lower rating on the importance of preventative actions. While a range of prevention methods are implemented, active community involvement is highly important for successful Dengue prevention. Continual evaluation is necessary to make more immediate, long-term behavioral societal changes, and to maximize the economic resources spent on Dengue prevention. Rev. Biol. Trop. 62 (3): 859-867. Epub 2014 September 01.

Key words: dengue fever, Costa Rica, social communication, Aedes aegypti, arbovirus, KAP.
re-infected with the virus, leading to the spread of dengue across the Western Hemisphere once again (Gubler, 1998). Today the number of incidences of dengue is 30 times greater than it was 50yrs ago, with an estimated 390 million dengue infections annually (Bhatt et al., 2013) and 2.5 billion people living in areas of risk (WHO, 2009).

Exacerbated by increases in climatic temperatures, frequent intercontinental airline travel, booming, uncontrolled urbanization, inadequate municipal services, and vector adaptability, (Parks & Lloyd, 2004; Farrar et al., 2007; Miller, 2012) dengue is a pressing global health concern. Relatively successful, the country of Costa Rica remained free of dengue until an epidemic outbreak in 1993. Since 1993, infections have persisted and posed an increasing health threat in Costa Rica, and there is a severe gap in published dengue research in the country (Troyo, Porcelain, Calderón-Arguedas, Chadee, & Beier, 2006). In 2013 Costa Rica was declared to have the largest dengue epidemic since 1993, and by the end of August 2013 around 27,000 infections were reported.

*Aedes aegypti* maintains a lifestyle dependent on humans, and lives exclusively in human-populated areas (Trpis, Hausermann, & Craig, 1995). The female mosquito lays her eggs in clean water, and eggs can lay dormant for over a year in a dry state. While a number of options for diminishing the vector have been considered in the past 20yrs, including predation to kill the mosquitoes, fumigation, and shorter-lived organophosphate insecticides, these methods are not successfully executed and are difficult to manage without community participation. *Aedes* breeding is often propagated by the failure to remove breeding sites, such as old tires, flower vases, random garbage, and even pop bottle caps that should be properly disposed of. Studies show that in working to eliminate dengue, community education and involvement are crucial (Espinoza-Gómez, Hernández-Suárez, & Coll-Cárdenas, 2002; Parks & Lloyd, 2004; Martin & Prado, 2004; Nam et al., 2005), yet much effort is still needed to optimize community involvement (Toledo-Románi, Baly-Gil, Ceballos-Ursula, Boelaert, & Van der Stuyft, 2006).

In order to combat dengue and eradicate the vector, the Pan-American Health Organization, national health care system and the Ministry of Health of Costa Rica designed a plan in 2005. The plan, EGI (Spanish for Integrative Management Strategy), has five integrated components, one of which is social communication. A goal of social communication is to mobilize public participation in eliminating the vector; however, attaining and maintaining long-lasting behavioral changes in a community is a difficult task (Parks & Lloyd, 2004). It was reported that public education regarding dengue knowledge is relatively strong in Costa Rica, yet education on attitudes and beneficial practices has not been well provided (Solís, Quesada, & Gutiérrez, 2010).

This study aimed to evaluate and report on the current status of the residents’ knowledge, perceptions and actions in regards to dengue in the small, mid-Pacific coastal village Quebrada Ganado, Puntarenas, Costa Rica.

**MATERIALS AND METHODS**

**Study site:** The study site was Quebrada Ganado, Puntarenas, Costa Rica (hereafter referred to as Quebrada, or QG), (9.72°N - 84.63°W), with a population of 1,916 (967 males, 949 females) (INEC, 2011). Before the study began, QG had 40 confirmed and reported cases of dengue in the first 16wks of 2013 (Ministry of Health). The town has 754 residences including 583 occupied and 171 unoccupied (INEC, 2011). Ninety-nine percent of the houses (582/583) have internal running water, and 99% (577/583) have a television (INEC, 2011). In general, houses are very close together and space is concentrated, including a community center, soccer field, kindergarten, pharmacy, primary and secondary schools, and public health clinic. The most commonly-reported resident occupations are personal services, cleaning services, sales and construction (INEC, 2011).
Study design: To correlate resident knowledge and attitudes with their respective property, and to evaluate the behavior of QG citizens, only citizens at residential properties were interviewed. Our 25-item interview was adapted from a similar interview designed to aid the Ministry of Health (MH) in a 2004 study (R. Baxter, unpublished). All interviews took place in March and April, 2013. Interview procedures, including interviewee verbal consent protocol, were approved by the Ethics Review Board of the Associated Colleges of the Midwest, Chicago, Illinois.

Household inspections were carried out during March through May, 2013 by employees of the Vector Control sector of the Garabito Ministry of Health of Costa Rica. As part of the EGI branch of Entomological Surveillance, MH workers inspect properties for possible and positive Aedes breeding sites, and educate owners about dengue prevention.

All answers were directly recorded on an interview sheet. Independent of how many people were present in the house, only one person was asked to respond. All respondents were at least 18 years of age, lived in the house, and as often as possible, managed household decisions. To avoid influence by recent in-house education by MH employees, we interviewed 182 households with no previous recent MH visit. These properties were inspected after the interview. An additional 37 interviews were conducted directly after a MH inspection, and 45 interviews were conducted in households that had an inspection two months prior. Fifty-six interviews were conducted in households with no previous inspection, and no MH inspection data following the interview were available for these houses.

All data were entered in Microsoft Excel and rechecked. Dependency of type of water used for mosquito breeding with prior education by MH was tested with a contingency table. The relationship between Likert scale or other types of responses for various independent and dependent variables of interest were estimated by simple linear regression. Differences in mean Likert scale responses between various groupings of participants were estimated with one-way parametric ANOVA. Assumptions of homogeneity of variance were tested and posterior comparisons between means were carried out with LSD, Tukey or Scheffé tests. Statistical procedures followed Sokal & Rohlf (1995). Analysis of variance and linear regression analyses were conducted using Statgraphics Centurion XVI (Statpoint Technologies, Inc., 2011).

RESULTS

Table 1 and additional comments appear as Digital Appendices. The rest of the results are detailed below.

Sample demographics: Of the 19 city blocks in the study site, 18 blocks were visited and 320 citizens were interviewed. This included 242 (76%) females and 78 (24%) males. Median age was 40 with a range from 18 to 86. Twenty-three (7%) participants had no education, 167 (52%) had received education at the primary level, 102 (32%) at the secondary level and 28 (9%) up to university level. Fifty-three percent of participants were stay-at-home mothers or not employed, 14% were blue-collar workers (construction, packing, maintenance, fishing, among others), 13% worked in a restaurant, tourism, or as a cashier and the other 20% were employed in 10 other sectors including childcare, independent work and security. Sixty-five percent of those interviewed owned their home or paid mortgage and 35% rented.

Citizen history with dengue and familiarity: Sixty-one percent of participants reported someone in their house had been previously infected with dengue and 21 (11%) of these participants reported it to be “hemorrhagic”. According to the previous WHO guidelines for dengue classification, community members were more familiar with this terminology than with the newer classification of “severe dengue.” Thirty-nine percent had no household member with previous infection. Of those who
reported a previous infection in the house, an average of two people in the house had contracted dengue and on average one time ranging from a reported one to six times.

Participants were asked to list the symptoms of dengue fever, and 89% of participants could state three or more symptoms. We designated three or more symptoms as an adequate understanding of the symptoms of dengue. The most common symptoms mentioned were fever (285 participants, 89%), headache (209 participants, 65%), body pain (134 participants, 42%), vomiting (122 participants, 38%), and rash (105 participants, 33%). Of those who had an adequate understanding, participants mentioned up to nine symptoms (n=2); with a mean, median and mode of four symptoms. Participants with previous acquisition were more knowledgeable of the symptoms, (F=76.6; df=1, 318; p<0.0001, R²=19.41%).

Citizen knowledge: Three hundred fifteen (98%) participants knew that dengue is transmitted by a mosquito and 98% knew that the mosquito reproduces in stagnant water. It became evident that the town has common knowledge that mosquitoes reproduce in water, but they grossly generalize all mosquitoes, specifically those around dirty street water, to carry dengue. When this generalization became evident, we began asking participants (n=253) what kind of water is preferred by the mosquitoes that transmit dengue. As previously noted, participants were interviewed either before or after an inspection by the Ministry of Health workers (Table 1) (Appendix 1). Participants reported clean water more often if their house had been inspected by a Ministry of Health worker 5wks prior, compared with the participants with no previous house inspection (χ²=8.88; df=1; p=0.0029). Education from MH inspections had an effect on community knowledge 5 weeks later. When asked how to decrease the number of dengue infections in the community, 280 participants (88%) mentioned eliminating stagnant water (Table 2) (Appendix 1).

Community concerns: When asked for the “most severe problem in the community”, 20 people (6%) reported a lack of available appointments in the clinic, 12 people (4%) reported garbage thrown in the streets and river, nine (3%) reported poor management of water in the streets, five (1.6%) reported inconsideration for the environment, four (1.3%) reported non-efficient political organizations, and dengue or excess of mosquitoes was reported by 13 (5%). The most common answer was illegal drug use (29%, 94 people).

When asked for the “principal health problem in QG”, an overwhelming 160 (50%) participants reported dengue. Six (2%) reported their concern for contaminated, stagnant water in the streets, and four (1.3%) reported a plague of mosquitoes.

Citizen perceptions: When asked: How dangerous it is for a person to get infected with dengue? 157 (52%) respondents said it is very dangerous, 125 (42%) said it is dangerous, and 18 (6%) said it is not dangerous, or they did not know. Mean response for danger level was greater for respondents that had a house member with previous dengue infection (μ=2.52 on scale from 1-3) when compared to the mean response for respondents who had no house member previously infected (μ=2.32) (F=5.97; df=1, 298; p=0.015).

Two hundred twelve (71%) respondents reported that “actions on dengue prevention” are very important, 75 (25%) reported the actions are important, three (1%) reported the actions are not important, and 10 (3%) reported they did not know. Mean response for perceived “importance of actions on dengue prevention” were greater for participants with a previously infected house member (μ=2.74) than for those with no previous household infection (μ=2.46) (F=13.23; df=1, 298; p=0.0003). Similarly, respondents’ rating on the “importance of actions on dengue prevention” was greater for respondents who could name “three or more dengue symptoms” (μ=2.66) compared to those who could not (μ=2.38) (F=5.25; df=1, 298; p=0.0227). Similarly, those
who could name “three or more dengue symptoms” perceived a greater “danger of infection” ($\bar{x}=2.46$) than those who could not ($\bar{x}=2.29$) ($F=1.77; df=1, 298; p=0.18$).

**Citizen opinions on responsibility and effective dengue education in the community:** The last three questions in the interview were used to gather community views, opinions and advice on dengue prevention within their community. In response to the question: Who has the responsibility of protecting the community from dengue? 167 (52%) reported it was their responsibility entirely, 29% responded the community has responsibility along with other organizations, and 19% did not include the community in their response (Table 3) (Appendix 1).

We asked participants: How the community can be educated on dengue and its prevention? and 406 responses were received. Response frequencies from the 320 participant total were calculated as percentages (Table 4) (Appendix 1). Many comments on dengue education were also offered, and some of the most common comments were recorded (Table 5) (Appendix 1). We also asked: How community members can be convinced to eliminate breeding sites on their properties? (Table 6) (Appendix 1), and additional comments were recorded (Table 7) (Appendix 1).

**Citizen practices:** Based on 264 properties inspected by MH employees, all 264 properties (100%) had possible breeding sites. Mean number of possible breeding sites per house was 8.5 (median=7, mode=5, and ranged from 2-51 per house). Nine percent of the properties inspected had at least one breeding site positive for *Aedes aegypti* larvae or pupas ($n=24$). The quantity of positive breeding sites found on each property ranged from 0 to 6, and included a variety of receptacles, including small buckets, flowerpots, and appliances not in use. The maximum number of times a house member had been infected by dengue was positively related to the number of possible breeding sites at that house ($F=5.51; df=1, 261; p=0.0196; R^2=2.07$). Houses with more possible breeding sites had more positive breeding sites for *A. aegypti* larva/pupa ($F=142; df=1, 263; p<0.0001; R^2=35.17$).

**Citizen trends and predictive factors:** Having a previous infection or knowledge of dengue symptoms did not have a significant relationship with the number of breeding sites found during inspections. Among the knowledge and demographic factors collected, participant education level had the greatest relationship to number of positive breeding sites found in inspections (negative relationship) ($F=5.92; df=1, 262; p=0.0156; R^2=2.21$). The number of positive breeding sites was also greater in homes where a) the respondent rated a lower importance on the actions of dengue prevention ($F=5.08; df=1, 244; p=0.0251; R^2=2.05$) and b) the rating for dengue danger was lower ($F=5.08; df=1, 244; p=0.0223; R^2=2.13$).

**DISCUSSION**

As with other studies, most often women of the house were available for interviewing (de María Cáceres-Manrique, Vesga-Gómez, Perea-Florez, Ruitort, & Talbot, 2009; Zuleta et al., 2011). Based on our total sample size of 42.4% of the residences in QG, the majority of townspeople reported having had a dengue infection in their household (61%). Nine in ten citizens (90%) have an adequate understanding of the fever’s symptoms, indicating they understand dengue fever well, which is comparatively high (Panagos, Lacy, Gubler, & Macpherson, 2005). An overwhelming 98% understand that dengue is transmitted by a mosquito, but think erroneously that the mosquito proliferates in stagnant water. However, a portion of participants reported false positive dengue cases. They reported up to six distinct dengue infections, which is not possible with four serotypes. Citizens also had a defined confusion on the characteristics of the dengue vector. Previous studies have shown a similar trend where communities are familiar with
dengue and mosquitoes, but have a poorer understanding of the relationship between the mosquito, human behavior and transmission (Panagos et al., 2005).

Citizens were not well educated on the differences in mosquito niches, leaving them to perceive “a mosquito is a mosquito,” hence, all mosquitoes transmit dengue. For example, citizens were preoccupied that dengue was spread by mosquitoes that are often found in areas where Aedes does not reproduce. They failed to focus on the primary breeding sites the dengue vector prefers: receptacles or other items with solid, flat surfaces that hold clean water in residential areas. Previous studies similarly reported participants failed to recognize mosquitos in their house as the dengue vector (Pérez-Guerra, Seda, García-Rivera, & Clark, 2005; Torres-López, Guerrero-Cordero, & Salazar-Estrada, 2012). Reported social barriers include a lack of responsibility and a muted concern of the disease (Pérez, Zielinski, Vargas, & Clark, 2009), especially due to hazy information (Torres-López et al., 2012). We believe the inability to differentiate and identify practices between mosquitoes may lead to this lack of responsibility and a muted concern, as well as the failure to mobilize in QG.

The primary role communities play in reducing the risk for dengue is to remove receptacles on their property (San Martin & Prado, 2004), and the role of community participation needs to be unified within a community (Toledo-Romání et al., 2006). A blurry understanding of Aedes habits mutes the relevancy and urgency for community members to play their part. By comprehending the distinctions of the dengue vector, community members will understand the reason they must review their lawns diligently, and unify on their participatory role.

Regarding community views on dengue responsibility, eight in ten (81%) respondents said the community is at least partially responsible for dengue prevention; however, about 20 percent do not claim any responsibility. This is a concern because a mindset of responsibility and ownership leads citizens to act. Citizens reported the best ways to convince community members to eliminate stagnant water were through: campaigns, information on risks and dangers, talks and lectures and the work by the MH. That is, the number one way to convince members to act is through more information and education.

The next most popular response was that contracting dengue is the only way to convince members to act; indicating personal experience is the best teacher. About one in three respondents expressed doubts in convincing other community members to take preventative actions. This stems from a long-standing culture that community members expect their neighbor will not take action and remove breeding sites on their property. For example, in the town it is “normal” to litter, therefore watching a neighbor throw trash on the street is acceptable. In order to change this norm, each member has to not only take individual ownership, but collaborate with others. This attitude of pointing the finger at one’s neighbors is not unique to this community (Pérez-Guerra et al., 2005, Torres-López et al., 2012). But members need to take ownership, challenge norms, and even expand their personal responsibilities. Some community members are physically unable to independently review their lawns adequately, such as the elderly. These people need family members or neighbors to step in for them.

Respondents also suggested dengue education needs to be frequent, continual and non-stop, because community members are human and therefore tend to “forget” if information is not brought up often. This report reflects one of the tenets of Communication for Behavior Impact (COMBI) put out by the WHO: sustained appropriate advertising which is massive, repetitive, intense and persistent (Parks & Lloyd, 2004), which has been supported in other studies as well (Khun & Manderson, 2007).

Regarding MH vector control inspections, MH workers inspect properties to remove and prevent Aedes breeding sites, and to educate citizens on dengue and its prevention. The MH

visits and inspections were of the most common suggestions on how to educate and even to convince community members on dengue and its prevention. Other communities have similarly requested house visits (Zuleta et al., 2011) to explain specific control measures (Pérez-Guerra et al., 2005). The use of dialogue has been stressed in the transition from critical understanding to critical action (Freire, 1973), and a house visit provides a great opportunity for one-on-one dialogue with MH educators and residents.

House visits and family education require many trained vector control workers, and a lot of time. Additionally, some citizens report that the MH has abandoned their position in dengue control because they fumigate much less than before. The request for fumigation has been reported in other communities as well (Pérez-Guerra et al., 2005). However, an over-dependence on fumigation or repeated house visits diminishes the community’s ownership and perceived responsibility to review their own properties for breeding sites. There is a misunderstanding that fumigation equals support in fighting dengue. This aim should be openly communicated with community members so they do not get the incorrect impression that the MH is retracting its position or support in dengue control by decreasing the frequency of fumigation.

Regarding target audience characteristics to consider, dengue perceptions have been shown to vary based on previous acquisition (Pérez et al., 2009). We similarly found that respondents who have had a dengue infection in the household perceive dengue to be more dangerous, and they consider the actions of prevention to be more important. Respondents who were familiar with dengue symptoms were also stronger supporters of preventative actions. This indicates if people are more aware of dengue symptoms, they may be more inclined to prevent the disease. This supports the educational goal to highlight the symptoms of dengue and risks it poses to an individual (Pérez et al., 2009).

To avoid “preaching to the choir,” dengue education needs to target the right audience. According to our findings, we compiled the following characteristics which suggest the target audience should include people who: do not live with anyone previously infected by dengue, do not understand the symptoms of dengue, have more frequent infections in the household (relates to more possible breeding sites), have a low education level (Rosenbaum et al., 1995, Syed et al., 2010), rate the importance of actions on dengue prevention lower (which relates to more positive breeding sites), rate dengue to be less dangerous (which relates to more positive breeding sites), and have more possible breeding sites on their property (which relates to more positive breeding sites).

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RESUMEN

Conocimientos, percepciones y prácticas para prevenir el dengue en un pueblo costero del Pacífico central de Costa Rica. Entre las enfermedades virales transmitidas por artrópodos, el dengue es la más mortal y de difusión más rápida en el mundo. Es transmitida por los mosquitos Aedes, y está presente en más de 125 países. En 2013, Costa Rica tuvo el mayor porcentaje de casos de dengue desde la reintroducción del virus al país en 1993. Este estudio evaluó el conocimiento de 320 miembros de la comunidad costera de Quebrada Ganado, Costa Rica, quienes dieron sus opiniones acerca de cómo mejorar la educación y movilizar la comunidad en la prevención del dengue durante marzo y abril 2013. Además, mediante inspecciones de propiedades se trató de hallar conexiones entre el conocimiento u opiniones y la presencia de criaderos de Aedes. Aunque los habitantes sabían que el virus es transmitido por los mosquitos que se reproducen cerca del agua, no reconocían los hábitos del mosquito y lo confundían con otros mosquitos. El 81% acepta la responsabilidad por la prevención del dengue. Sugerencias para mejorar la educación incluyeron: información continua y frecuente sobre los riesgos, peligros y daños a la salud.
características relacionadas con las propiedades que tenían más criaderos fueron: menor nivel de educación, mayor frecuencia de infecciones con dengue, menor entendimiento de los síntomas del dengue, menor calificación dada a los peligros del dengue y menor calificación dada a la importancia de las acciones preventivas. Mientras se pone en práctica una gama amplia de métodos de prevención del dengue, la participación activa de la comunidad es sumamente importante para el éxito de estas actividades. La evaluación continua es necesaria para lograr cambios inmediatos y a largo plazo en el comportamiento social y para aprovechar mejor los recursos económicos invertidos en la prevención del dengue.

**Palabras clave:** dengue, Costa Rica, comunicación social, *Aedes aegypti.*

**REFERENCES**


Incidence and risk factors for cognitive impairment in rural elderly populations in Costa Rica

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Abstract: Risk factors for the onset of cognitive impairment in Costa Rica are not well understood, despite a substantial elderly population stemming from a higher than average life expectancy for the western hemisphere. To investigate the risk factors that predict the onset of cognitive impairment in the rural elderly of Costa Rica, a modified version of the Mini Mental State Exam—designed for illiterate populations—was administered to 90 elderly inhabitants of San Carlos, Alajuela, Costa Rica between April and May of 2011. Subsequently, each participant took a structured interview assessing viability of risk factors and behaviors potentially contributing to a diagnosis of cognitive impairment. Results showed strong dependencies between age (p=0.0001), education level (p=0.0095), the ability to read (p=0.0001) and write (p=0.0153), frequency of reading (p=0.0011), use of puzzles and mind games (p<0.0001), vocation (p=0.0225), area of residence (p=0.0001), comorbid mental diseases (p=0.0005), history of stroke or brain trauma (p=0.0104), urinary or renal problems (p=0.0443), consistent cooking practices (p=0.0262) and number of living companions (p=0.0299) in susceptibility for developing cognitive impairment. The study concluded that high intellectual use, or lack thereof, during the lifetime of a person was a predictor for cognitive status later in life. In addition, comorbid mental disorders, including neurological trauma due to stroke, impeded normal cognitive function. Future research should examine incidence and risk factors of cognitive impairment in urban, more educated populations. Rev. Biol. Trop. 62 (3): 869-876. Epub 2014 September 01.

Key words: cognitive impairment, dementia, education, elderly, mini mental state exam, Costa Rica.

Although widely applicable to the Costa Rican public health sector, risk factors for cognitive impairment in Costa Rica are not well understood. With a substantial elderly population stemming from a higher than average life expectancy for the western hemisphere, and a portion of this population living in rural areas of the country, incapacitation attributable to cognitive malfunction in the rural elderly is prevalent (Cáceres-Rodas, 2004). Cognitive impairment is a key indicator for a diagnosis of dementia, defined as a progressive, global or local loss of proper mental function (Yen et al., 2010). The primary symptoms associated with dementia are characterized in the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision (DSM-IV TR), and include memory loss, attention deficits, a loss of problem solving skills, mood and attitude changes, and trouble producing and understanding language (American Psychiatric Association, 2000). Dementia is a characteristic symptom of several mental health disorders also diagnosable through DSM-IV TR criteria, including Alzheimer’s disease (AD). In fact, people diagnosed with Mild Cognitive Impairment (MCI) are at significant risk for developing AD and other age-related neurodegenerative disorders (Belleville, Sylvain-Roy, de Boysson, & Ménard, 2008; Li et al., 2011; Petersen et al., 1999; Petersen et al., 2001).

With the current rate at which globally applicable biomedical research is advancing, the average human life span is dramatically
increasing. Costa Rica is no exception. According to the 1984 census, there were 158,144 people over the age of 60 in Costa Rica, comprising approximately 6.5% of the population. The 2000 census revealed an increase to 301,474 people over the age of 60, making up 7.9% of the Costa Rican population. Current projections for the year 2015 place the estimated elderly population at 557,076, which would correlate to more than 11.1% of the population (Cáceres-Rodas, 2004; Centro Centroamericano de Población, 2011). Additionally, portions of Costa Rica have been designated a Blue Zone, which are defined as special areas of the world with high numbers of living centenarians (Buettner, 2010). With this rapidly aging population, it is becoming increasingly important to assess the prevalence of cognitive impairment, identify risk factors associated with such impairment, and educate the public on ways to preserve their mental status as they age.

Numerous studies in other countries have shown that age, gender, education level, cardiovascular health, daily nutritional value, living situation, pollution, consumption of tobacco and alcohol, and other comorbid mental and non-mental diseases are significant risk factors for a diagnosis of cognitive impairment (Yen et al., 2010; Heun & Hein, 2005; Lee, Hong, Cheong, & Oh, 2009; Sánchez-Rodríguez, Santiago, Arronte-Rosales, Vargas-Guadarrama, Mendoza-Núñez, 2006; van Exel et al., 2002). However, a unique climate, economy, culture, location, and greater than average life expectancy contribute to risk factors specific to Costa Rica. Cáceres-Rodas (2004) explored the relationship between physical health and mental health in the outer lying rural and quasi-urban parts of the Central Valley of Costa Rica. This study, though, did not specifically investigate behavioral, social, or cultural risk factors for cognitive impairment.

The objectives of this study were to: a) individually assess elderly patients in Florencia and Ciudad Quesada for differences in the incidence rate of cognitive impairment using the Mini Mental State Exam; b) assess potential risk factors for cognitive impairment in a structured interview following the Mini Mental State Exam; and c) estimate the correlations between risk factors and presence and severity of cognitive impairment.

MATERIALS AND METHODS

Study site: This study was carried out in two locations: Florencia, San Carlos, Alajuela, Costa Rica (10°22’0” N and 84°29’0” W) and Ciudad Quesada, San Carlos, Alajuela, Costa Rica (10°20’0” N and 84°26’0” W) (Centro Centroamericano de Población, 2011).

Study population: The study population was anyone over the age of 60 as defined as elderly for rural research by the World Health Organization (2011). All participants fulfilled one or more of the following three criteria to create a random sample: a) being part of a non-residential, elderly support group in the above districts; b) living in an elderly institution providing around-the-clock medical and social assistance; c) in their home when interviewed by the researcher in neighborhoods of Ciudad Quesada and Florencia, San Carlos, Alajuela, Costa Rica.

Interview criteria: Human participants and treatment during the interview was in accordance with the International Ethical Guidelines for Biomedical Research Involving Human Subjects as set forth by the Council for International Organizations of Medical Sciences in collaboration with the World Health Organization in 2002. Prior to the interview, all participants signed a standard consent form explaining information about the purpose of the study, the rights of the participant, and the risks of involvement. The form was read to each participant orally to ensure that all participants fully understood the implications of their participation. Literate subjects signed their name and illiterate participants supplied their fingerprint to confirm their consent.

Subsequently, a modified version of the Mini Mental State Exam (MMSE) was administered to determine whether or not there was
an incidence of cognitive impairment and if so, to what degree. This rural population had a high percentage of illiterate and uneducated elderly persons. As such, an adjusted version of the MMSE designed for uneducated and illiterate populations was utilized (Kabir & Herlitz, 2000).

After administration of the MMSE, all patients underwent a structured interview survey aimed at assessing risk factors for cognitive impairment. Both of these examinations were administered orally in an interview format.

**Assessment survey variables:** Variables examined in the risk-factor assessment survey included: a) mental exercises—participant regularly completes puzzles, word searches, crossword puzzles, plays cards, dominoes, or other mental puzzles or exercises; b) location of residence—participant lived only in a rural region, in both a rural and urban region, or only in an urban region during their life; c) ability to read—a self report of whether the participant can read well, can barely read, or cannot read at all; d) age—participant is 60-70yrs old, 71-80yrs old, or 81+yrs old; e) comorbid mental issues—self report of whether the patient has been diagnosed with mental or neurological conditions such as depressed continuously for more than one month, issues with anxiety or nervousness, or takes or has taken treatments for mental problems; f) frequency of reading—participant reads everyday, reads 2-6d of the week, or reads less than one time per week; g) formal education—participant received no formal education, completed 1-2 grade, completed 3-4 grade, completed 5-6 grade, or received more than six years of formal education; h) stroke—participant has had a stroke, or another form of vascular or cerebral trauma; i) ability to write—participant can write more than their name, or the participant can write only their name, or the participant cannot write; j) vocation—participant was an agricultural worker, was a home-maker, or worked in business, commerce, or city-oriented jobs; k) cooked for themselves—participant was responsible for preparing their own meals, or does not prepare their own meals; l) living companions—participant lives alone, lives with 1-3 others, or lives with four or more other people; m) urinary problems—participant suffers from urinary problems, or does not suffer from urinary problems; n) tv watching time—participant watches less than one hour of television per day, 1-3hrs of television per day, or more than 3 hours of television per day; o) diabetes—participant suffers from either Type I or Type II Diabetes, or does not suffer from diabetes; p) living siblings—participant has three or fewer siblings still living, or has four or more living siblings; q) cholesterol—participant is currently suffering from high cholesterol, has suffered from high cholesterol in the past, or does not and has never suffered from high cholesterol; r) number of children—participant’s has reared three or fewer children, or has reared four or more children; s) number of surgeries—participant has had 2 or more surgeries, or has had 1 or fewer surgeries; t) death of a child—participant has reared a child who has subsequently died, or all of the children of the participant are still living; u) colon problems—participant suffers from any number of intestinal problems including, but not limited to, colitis, or the participant does not suffer from colon problems; v) smoker or past smoker—participant currently smokes or has smoked in the past, or the participant has never smoked; w) family mental issues—someone in the participants family has suffered from mental disease, stroke, or brain trauma, or no one in the family has suffered from mental or neurologic problems; x) gender—participant is male or female; y) eats dinner—participant eats dinner regularly, or participant does not eat dinner; z) pregnancy frequency—female only: participant has been pregnant four or fewer times, or five or more times; aa) drinker or past drinker—participant currently or habitually consumed alcoholic beverages now or in the past, or has never consumed alcoholic beverages; ab) tv companions—participant watches TV alone, or watches with other people; ac) heart attack—participant has had a heart attack, or the participant has never had a heart attack; ad) hypertension—participant is currently...
suffering from hypertension, or has suffered from hypertension in the past; ae) cardiovascular—participant self reports that a doctor has previously told them that they have heart problems, or that they have never been informed of heart problems; and af) gastric problems—participant suffers from any number of gastric problems including, but not limited to, gastritis, or does not suffer from gastric problems.

The MMSEs were scored, and overall prevalence of cognitive impairment was estimated. Participants were considered cognitively impaired by scoring below 25 out of 30 possible points on the MMSE. A score of ≤9 points indicated severe cognitive impairment, a score of 10-20 points indicated moderate cognitive impairment, and a score of 20-24 indicated mild cognitive impairment.

This measured independence between individual risk factors and incidence of cognitive impairment through contingency tables. Each severity category was not analyzed with each variable. Only the overall incidence (all severity categories combined) or lack of cognitive impairment was measured against the individual risk factor variables. Statistical tests followed Sokal & Rohlf (1995) and were run with Statgraphics Centurion XVI.I software (Statpoint Technologies, Inc., 2011).

RESULTS

This study included a total of 90 participants. One participant underwent administration of a non-modified MMSE for illiterate populations, was unable to be re-tested with an unbiased form of the exam, and was thus excluded from analysis. Of those included, 60.6% were women. Mean age of participants was 76.8±8.1 (Mean±S.D.) years; 33.7% were over the age of 80. In terms of educational status, 5.6% received more than a primary education, with 41.6% of participants having completed the second year of formal primary education or less. The majority of the study population (96.6%) was born in Costa Rica. Overall incidence of cognitive impairment (mild, moderate and severe combined) was 48.3%. Of those impaired, 48.8% were mildly impaired, 48.8% were moderately impaired, and 2.3% were severely impaired.

Results of bi-variate contingency table analysis of independence between individual risk factor variables and the incidence of cognitive impairment are summarized in table 1. Specific elucidation of three statistically significant risk factor variables—formal education, vocation, and age—can be seen in a categorical breakdown in Figs. 1-3, respectively.

There was a significant dependency of the selection group sampled on the incidence of cognitive impairment ($X^2 = 8.9$, df=3, $p=0.030$).

Fig. 1. Frequency of participants and their obtained educational level as it corresponds to mental state, April-May 2011, San Carlos, Costa Rica.

Fig. 2. Frequency of participants and their vocational type as it corresponds to mental state, April-May 2011, San Carlos, Costa Rica.
Residents of the Hogar de Ancianos had the most impairment (77.8%), followed by Ciudad Quesada (42.3%), and then Florencia (41.7%). The lowest proportion of impairment was noted in the group collected house to house (33.4%).

**DISCUSSION**

Nearly all of the significant risk factors leading to increased impairment shared the common theme of lack of mental stimulation. Higher levels of brain input and stimulation appeared to exhibit neuroprotective effects as the study population aged. Acquiring and/or sustaining formal education by frequently reading, writing, and completing puzzles also appeared to aid in staving off brain atrophy. Additionally, both a city-oriented vocation that requires enhanced daily thought processing and a home in a suburban or city atmosphere where more visual, auditory, and interpersonal stimulation exists had seemingly similar effects.

**TABLE 1**

<table>
<thead>
<tr>
<th>Risk Factor Variables</th>
<th>X² (Chi-Squared)</th>
<th>Degrees of Freedom</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mental Exercises</td>
<td>18.417</td>
<td>1</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Location of Residence</td>
<td>20.509</td>
<td>2</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Ability to Read</td>
<td>17.896</td>
<td>2</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Age</td>
<td>19.297</td>
<td>2</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Comorbid Mental Issues</td>
<td>12.105</td>
<td>1</td>
<td>0.0005*</td>
</tr>
<tr>
<td>Frequency of Reading</td>
<td>13.555</td>
<td>2</td>
<td>0.0011*</td>
</tr>
<tr>
<td>Formal Education</td>
<td>13.406</td>
<td>4</td>
<td>0.0095*</td>
</tr>
<tr>
<td>Stroke</td>
<td>6.565</td>
<td>1</td>
<td>0.0104*</td>
</tr>
<tr>
<td>Ability to Write</td>
<td>5.822</td>
<td>1</td>
<td>0.0153*</td>
</tr>
<tr>
<td>Vocation</td>
<td>7.591</td>
<td>2</td>
<td>0.0225*</td>
</tr>
<tr>
<td>Cooked for Themselves</td>
<td>4.945</td>
<td>1</td>
<td>0.0262*</td>
</tr>
<tr>
<td>Living Companions</td>
<td>4.715</td>
<td>1</td>
<td>0.0299*</td>
</tr>
<tr>
<td>Urinary Problems</td>
<td>4.044</td>
<td>1</td>
<td>0.0443*</td>
</tr>
<tr>
<td>TV Watching Time</td>
<td>2.740</td>
<td>1</td>
<td>0.0979</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.927</td>
<td>1</td>
<td>0.1651</td>
</tr>
<tr>
<td>Living Siblings</td>
<td>1.857</td>
<td>1</td>
<td>0.1730</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1.829</td>
<td>1</td>
<td>0.1762</td>
</tr>
<tr>
<td>Number of Children</td>
<td>1.084</td>
<td>1</td>
<td>0.2979</td>
</tr>
<tr>
<td>Number of Surgeries</td>
<td>0.934</td>
<td>1</td>
<td>0.3337</td>
</tr>
<tr>
<td>Death of Child</td>
<td>0.757</td>
<td>1</td>
<td>0.3842</td>
</tr>
<tr>
<td>Colon Problems</td>
<td>0.685</td>
<td>1</td>
<td>0.4078</td>
</tr>
<tr>
<td>Smoker or Past Smoker</td>
<td>0.440</td>
<td>1</td>
<td>0.5072</td>
</tr>
<tr>
<td>Family Mental Issues</td>
<td>0.440</td>
<td>1</td>
<td>0.5072</td>
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<tr>
<td>Gender</td>
<td>0.435</td>
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<td>0.5097</td>
</tr>
<tr>
<td>Eats Dinner</td>
<td>0.116</td>
<td>1</td>
<td>0.7332</td>
</tr>
<tr>
<td>Pregnancy Frequency</td>
<td>0.092</td>
<td>1</td>
<td>0.7613</td>
</tr>
<tr>
<td>Drinker or Past Drinker</td>
<td>0.054</td>
<td>1</td>
<td>0.8163</td>
</tr>
<tr>
<td>TV Companions</td>
<td>0.036</td>
<td>1</td>
<td>0.8499</td>
</tr>
<tr>
<td>Heart Attack</td>
<td>0.028</td>
<td>1</td>
<td>0.8675</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.020</td>
<td>1</td>
<td>0.8867</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>0.015</td>
<td>1</td>
<td>0.9012</td>
</tr>
<tr>
<td>Gastric Problems</td>
<td>0.014</td>
<td>1</td>
<td>0.9050</td>
</tr>
</tbody>
</table>

*Denotes statistical significance at the p<0.05 level.
Numerous studies have found strong trends suggesting education level as a predictor for the onset of cognitive impairment, and that participation in life-long intellectual activity can be an important factor in staving off such symptoms (Callahan et al., 1996; Kliegel, Zimprich, & Rott, 2004; Marcopulos, McLain, & Giuliano, 1997; White et al., 1994). Similarly, intellectual stimulation can be seen as a technique to attenuate the harsh effects of cognitive impairment when it acts as a characteristic symptom of neurologic disorders such as multiple sclerosis (Sumowski, Wylie, Chiaravalloti, & DeLuca, 2010). As such, it is becoming clearer that higher intake of life-long intellectual stimuli is essential for resiliency in cognitive status, regardless of age or comorbid disease.

Regarding implications of brain traumas in impairment, not surprisingly, patients who had previously sustained brain trauma, whether in the form of stroke, or other, were significantly more impaired than their non-affected counterparts. A stroke is any interruption of blood supply to the brain, either by blockage or bursting (PubMed Health, 2011). The implications of a stroke, according to the same source, can cause lasting brain damage and mal-effects in speech production and comprehension, movement, bladder and bowel problems, issues with cognition and memory, among others. Furthermore, only about half of stroke patients regain enough function to independently care for themselves (PubMed Health, 2011). Thus, it is logical that stroke survivors tested significantly for increased cognitive impairment.

Regarding implications of dangerous behaviors in impairment, behaviors such as chronic smoking and drinking showed no significant effect on cognitive impairment in this study, debate still exists about the roles that smoking and alcohol consumption play in general cognition and incapacity (Hill, 1989; Reitz, Luchsinger, Tang, & Mayeux, 2005; Kalmijn, van Boxtel, Verschuren, Jolles, & Launer, 2002).

Gender played an insignificant role as an indicator for cognitive impairment in this study. Interestingly, Yen et al. (2010) reported females at higher risk for cognitive impairment. However, van Exel et al. (2001) showed women less prone to the development of cognitive impairment. Obvious debate exists, prompting the necessity for unbiased sampling. The present study is prone to a similar type of sample bias given the gender demographics of the sample population. In this sample population, greater than 60% of participants were female. The majority of male participants were interviewed in the Hogar de Ancianos, a hospital-type institution where more than 70% of residents interviewed exhibited cognitive impairment. Thus, with a higher than representative sample of impaired males, and a larger than 60% sample of females, it is possible that the sample bias contributed to a non-significant role of gender in cognitive impairment.

Additionally, the majority of health-oriented comorbidities in this study did not appear as significant indicators for a diagnosis of cognitive impairment. Significant correlations were previously reported for diabetes and cardiovascular disease as predictors of cognitive impairment (Yen et al., 2010; van Exel et al., 2002). However, the study population assessed in Yen et al. (2010) was nearly 10 yrs younger on average than in the present study. Further, van Exel et al. (2002) implemented the use of
electrocardiogram technology, and in-depth analysis of patient charts. Each of these methodological disparities can contribute to differences in findings.

Finally, self-reported data often provides a skewed view of the medical history of the patient. Patients may not understand exactly what a doctor is saying during consultations, especially in a highly uneducated, rural population. Thus, readers must take into account the means by which the data was collected when generalizing about the apparent insignificance of existing health comorbidities.

ACKNOWLEDGMENTS

We thank Chris Vaughan and Michael McCoy for their tireless efforts to make this project a success, help on statistical analysis, and manuscript edits. We also thank Olga Mora, Albino Riveras, and Dayma Morales, directors and overseers of the elderly institutions in San Carlos de Alajuela, Costa Rica. And finally, thanks are given to all study participants who selflessly shared their stories, insights, fears, and lives during this experience.

RESUMEN

Incidencia y factores de riesgo para la discapacidad cognitiva en poblaciones rurales de tercera edad en Costa Rica. Los factores de riesgo asociados con el inicio de la discapacidad cognitiva en Costa Rica son poco comprendidos, a pesar de la existencia de una considerable población de la tercera edad que ha resultado de una expectativa de vida mayor que el promedio en el hemisferio occidental. Para investigar los factores de riesgo que indican el inicio de la discapacidad cognitiva en adultos mayores de zonas rurales en Costa Rica, se les administró una versión modificada del Mini Examen del Estado Mental diseñado para poblaciones analfabetas a 90 adultos mayores residentes en San Carlos de Alajuela, Costa Rica, entre abril y mayo 2011. Posteriormente cada participante recibió una entrevista estructurada para evaluar la viabilidad de los factores de riesgo y comportamientos que podrían contribuir a un diagnóstico de discapacidad cognitiva. Los resultados mostraron una fuerte relación de dependencia entre la edad (p=0.0001), el nivel educativo (p=0.0095), la capacidad de leer (p=0.0001) y escribir (p=0.0153), la frecuencia de lectura (p=0.0011), la utilización de rompecabezas y juegos de estimulo intelectual (p<0.0001), la vocación (p=0.0225), el lugar de residencia (p<0.0001), las enfermedades mentales comórbidas (p=0.0005), un historial de derrame o de trauma cerebral (p=0.0104), los trastornos urinarios o renales (p=0.0443), la preparación de alimentos en forma consistente (p=0.0262), el número de personas con quienes convive (p=0.0299) y la susceptibilidad de desarrollar la discapacidad cognitiva. El estudio concluyó que un alto nivel de uso del intelecto, o la falta del mismo, durante la vida es un indicador del estatus cognitivo en etapas más avanzadas de la vida. Además, se encontró que las enfermedades mentales comórbidas, como el trauma neurológico provocado por un derrame cerebral, impiden la función cognitiva normal. Se recomienda que futuras investigaciones examinen la incidencia y los factores de riesgo asociados con la discapacidad cognitiva en poblaciones urbanas de mayores niveles educativos.

Palabras clave: discapacidad cognitiva, demencia, educación, adulto mayor, mini examen del estado mental, Costa Rica.

REFERENCES


Knowledge of cervical cancer pathology of high school students in San Carlos, Costa Rica

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Abstract: In Costa Rica, cervical cancer is the second most common female cancer, despite accessibility of screening through the universal health care system. There is little understanding of knowledge levels of the adolescent population on cervical cancer, although this population is the most exposed to risk factors. This study sought to learn about male and female adolescent knowledge of preventative methods, infection acquisition, clinical manifestations and medical services. A total of 533 students from grades 7-12 from five public high schools in the rural San Carlos region participated in the study from March through April 2012. Students were found to lack knowledge, as only 30.8% of students stated that they knew what cervical cancer is. Additionally, a connection was lacking between cervical cancer concepts, as for example 75.2% of students had heard of Human Papiloma Virus, but only 33.9% of those students knew that HPV is related to cervical cancer. Age had a positive relationship with knowledge of main concepts (p≤0.001). More women than men had heard of cervical cancer and the Papanicolaou (p=0.025, p≤0.0001), but otherwise no significant difference in mean response between genders was found. Students were found to have a limited awareness of the educational cervical cancer campaign (7.7% of the student population) and the ability to go alone to the doctor to receive medical attention (30.6% of students). Additionally, the public education system does not require cervical cancer to be included in the present curriculum. Hence, as students lack education on prevention and risk factors, the majority of prevention responsibility falls only on the universal health care system to regularly perform Papanicolaou exams to detect pre-cancerous or cancerous changes. The findings indicated the importance of including cervical cancer in the sexual education curriculum of the public education system in Costa Rica to educate the at-risk population of preventative methods, infection development, clinical manifestations and medical services in order to lower the presence of cervical cancer. Rev. Biol. Trop. 62 (3): 877-886. Epub 2014 September 01.

Key words: adolescents, cervical cancer, Costa Rica, Papanicolaou, sex education.

In order to prevent cervical cancer one should limit exposure to Human Papilloma Virus, especially by avoiding the main risk factors: an early onset of sexual activity and an elevated number of sexual partners (American Cancer Society, 2011). Additional risk factors include sexually transmitted diseases, smoking, obesity, contraceptive medications and unprotected sexual intercourse (American Cancer Society, 2011).

Cervical cancer develops due to gradual changes in the cervix after persistent, repeated HPV infections (Feldman, Sirovich, & Goodman, 2011). About 90% of the infections naturally clear, yet to avoid HPV exposure or to receive a HPV vaccination is highly recommended (Maine, Hurlburt, & Greeson, 2011). The Papanicolaou (Pap smear) is the most common screening test, or method to detect treatable pre-cancerous formations (Feldman et al., 2011). Screening is recommended every other year for sexually active women at least 20yrs old (Feldman et al., 2011).

Symptoms of cervical cancer, particularly abnormal vaginal bleeding and pain during sexual intercourse, generally arise in the latest
stages of the disease (American Cancer Society, 2011). Development from localized to metastasized cancer typically takes 10-15yrs (RHO Cervical Cancer, 2009). The survival rate for localized cancer is 90%, yet only 16.5% for metastasized cancer (American Cancer Society, 2011). This emphasizes the importance of detecting cervical cancer early through the Papanicolaou before symptoms arise.

Cervical cancer is the second most common cancer facing Costa Rican women (Aguado, Beauvais, Byrne, & Gacic-Dobo, 2010). Every year, an estimated 403 women are positively diagnosed and 158 die due to cervical cancer, in a population of over 2 million (Aguado et al., 2010; Instituto Nacional de Estadística y Censo [INEC], 2011). Screening methods are available for this preventable disease through Costa Rica’s universal health care system (Kivistik, Lang, Baili, Antitila, & Veerus, 2011). However, only an estimated 44.8% of Costa Rican women are yearly screened and 17% of women over 65yrs-old have never received a Pap smear (Aguado et al., 2010). Meanwhile, 52% of the sexually active Costa Rican population faced the onset of sexual activity between 14 and 17yrs of age, putting them at a greater risk of contracting cervical cancer (Picado, 2007).

Few studies have investigated the level of cervical cancer knowledge of males, teenagers or Latinos. A South African study focused on the knowledge of males of cervical cancer, found an extremely limited level of knowledge towards the disease and risk factors (Maree, Wright, & Makua, 2011). In another study, Nigerian women did not utilize screening exams, as they lacked knowledge of the disease and screening accessibility (Nwankwo, Aniebue, Aquwa, Anarado, & Aqunwah, 2011). Another South African study on the female population found basic preventative education should be given, in a manner appropriate to the cultural context (Francis et al., 2011). In socially-deprived areas of the United States acceptance of the Papanicolaou exam is lacking and these women not only were found to have a limited knowledge, but negative attitudes towards screening (Logan & Mcifatrick, 2011).

The “Caja Costarricense de Seguro Social” (CCSS), the universal health care system of Costa Rica, works to prevent cervical cancer through education and conducting the Papanicolaou (A. Muñoz, personal communication, April 16, 2012). One week during each October or November is dedicated to the “Semana de Salud” (“Health Week”). During this time, stands are placed outside the EBAIS, the local public clinics, each with different health themes. One stand is dedicated to cervical cancer and is geared towards teenagers, as they are the most vulnerable population since they are less likely to use condoms. They also work to regularly screen the female population.

Each adolescent that comes to the clinic receives a “risk classification.” When a patient indicates they are sexually active, the doctor speaks with the patient without their parents. In addition to receiving a discussion about reducing risk factors and receiving free contraceptives, females are advised to schedule a Pap smear for another day when they can come alone.

The objectives of this study were to measure: a) the level of knowledge of the adolescent population concerning: preventative measures, infection development, metastasis and available medical services; b) the level of knowledge related to age, gender and school type; c) other potential barriers inhibiting women from receiving screening tests.

**MATERIALS AND METHODS**

**Study area:** The study took place in Costa Rica in the province of Alajuela, within the towns of Aguas Zarcas, Río Cuarto, La Palmera and Venecia. Aguas Zarcas (10°20′N - 84°26′W) and Venecia (10°22′0″ N - 84°17′0″ W) are located 15km and 28km, respectively, from Ciudad Quesada, the largest city of the San Carlos region (Souza, 2011). Aguas Zarcas is 97km North of San José, the capital of Costa Rica (Souza, 2011). Aguas Zarcas has an estimated population of 13 651 residents (INEC, 2011). There are three high schools in the Aguas Zarcas district, including one in La...
Palmera. Venecia has an estimated population of 7,394 residents (INEC, 2011). There are four high schools within the Venecia district, including one school in Río Cuarto.

**Study population:** The study included public high school students from five schools within the Venecia and Aguas Zarcas school districts. Only students from grades 7-12 who consented to research were eligible to participate. High school sizes ranged from approximately 200 to 1,200 students (Table 1). A total of 533 students participated. The technical high schools ranged from grades 7-12, while academic high schools ranged from grades 7-11.

**Procedure/Creating questionnaire:** First, we created a questionnaire to analyze the level of knowledge of cervical cancer of male and female adolescents. To test for a basic level of understanding, we included initial questions pertaining to cervix identification, mortality and symptoms, as well as stating whether or not they know what cervical cancer is. Additionally, we added a series of questions about risk factors, HPV and infection acquisition, to measure the understanding of students of cervical cancer prevention.

We asked students to state whether they had heard of an educational cervical cancer campaign in their community or not, and to assess the level of its presence. We also included questions to measure the knowledge of teenagers about the medical services available, such as the Papanicolaou and whether unaccompanied doctor visits were possible. The final question asked students to identify potential barriers to going alone to the doctor.

**Receiving approval:** Before beginning research, we received approval from the St. Olaf Institutional Review Board. Then we received approval from the: a) regional director, Lic. Oscar Oviedo Valerio, at the Ministry of Education in Ciudad Quesada; b) district supervisor of Venecia, Rodolfo González, to receive permission to work within the Venecia school district; c) Rodolfo Salas, the district supervisor of Agua Zarcas, and d) high school principals.

**Initial field research:** We randomly chose classes to start distributing parental consent forms. We gave each class a brief explanation of the study, along with instructions to have their parent/guardian sign the consent form that night. We recorded each class section visited and their class schedule for the next day. The following day, we returned to the same class sections to distribute a student consent form to minors that returned the signed parental consent form and to those at least 18yrs old. We then administered the questionnaire to the students with both completed forms. We transferred the questionnaire results onto an electronic data sheet. We returned a third day to the same class sections to distribute questionnaires to any additional students who had brought the signed parental consent form. We repeated this process with every class section possible.

For most questions we used a condensed Likert scale with 0 and 1; 0= an incorrect or negative response and 1= a correct or positive response. We constructed frequency histograms of median answers with Microsoft Excel. Relationships between several dependent variables from questionnaire responses

![TABLE 1](image)

**TABLE 1**

Age and total number of students participating in the study for each age sampled. San Carlos, Costa Rica. March-April 2012

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Number of Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>44</td>
</tr>
<tr>
<td>13</td>
<td>82</td>
</tr>
<tr>
<td>14</td>
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<td>3</td>
</tr>
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<td>22</td>
<td>1</td>
</tr>
</tbody>
</table>
with age of respondent (independent variable) were estimated with simple linear parametric regression. Dependencies of responses between pairs of certain questions were estimated with contingency tables. Differences between mean responses of questions grouped by several participant characteristics were carried out with one-way, parametric ANOVA. Homogeneity of variance was tested before applying parametric ANOVA. Statistical procedures followed Sokal and Rohlf (1995). Statistical tests were run with Statgraphics Centurion XVI (Statpoint Technologies, Inc., 2011).

**Return to the field:** Following data analysis, we returned to San Carlos to discuss the findings and leave copies of the final paper with the medical doctor of EBAIS 2 of Venecia, the regional director in Ciudad Quesada, as well as the district supervisors, principals and interested teachers of each high school. We additionally left the answers to the questionnaires at each high school with enough copies for each teacher and class section, so the students could receive the correct information.

**RESULTS**

**Participants:** A total of 533 students completed the questionnaire from the five high schools studied. Participants ranged from 12-22yrs old and from grades 7-12 (Table 1). A total of 315 respondents were identified as females, and 216 identified as males.

The individual return rates of each school varied from 13.8% to 39.5%. The overall return rate was 27.5%. The technical schools had an overall return rate of 14.3%, while the academic schools had an overall return rate of 32.0%, excluding the Colegio Técnico de Aguas Zarcas, as not all of the classes were reached.

**Preventative measures:** Only 7.7% of students had heard of the presence of an educational cervical cancer campaign in their community. A total of 30.8% of students stated they knew what cervical cancer is, with the mean response for females ($\bar{x}=0.35$) greater than for males ($\bar{x}=0.25$) ($F=5.04$, d.f.=1, $p=0.025$; n=529). We found that 41.5% of the students knew cervical cancer is preventable. In identifying the two key risk factors, 30.8% of students identified an early onset of sexual activity and 37.1% identified an elevated number of sexual partners. A total of 92.1% of students identified at least one correct risk factor.

A total of 67.9% of students had heard of the Papanicolaou (Table 2). There was a strong positive relationship between age and likelihood of hearing of the PAP ($F=27.1$, d.f.=1, $p≤0.0001$, $R^2=4.88\%$; n=528, Table 3, Fig. 1). Additionally, mean female response ($\bar{x}=0.73$)

![Fig. 1. Relationship between student age (12-22 years old) and response when asked, “Have you heard of the Papanicolaou (PAP)?” Values of “0” and “1” represent responses of “no” and “yes”, respectively. Equation is: “Heard of PAP?”=-0.0544+0.0472*Age. Concave lines are 95% confidence intervals of the estimated line. San Carlos, Costa Rica. March-April 2012.](image-url)
was greater than male response ($\bar{x}_{\text{male}}=0.60$) ($F=9.37$, d.f.=1, $p=0.0023$; $n=528$). There also was a positive dependency between knowing cervical cancer is preventable and having heard of the Papanicolaou ($X^2=9.22$; d.f.=1; $p=0.0024$).

**Infection development:** We found 75.2% of the students had heard of Human Papilloma Virus (Table 2). A strong positive relationship existed between age and likelihood of hearing of HPV ($F=61.9$, d.f.=1, $p<0.0001$, $r^2=10.5$%; $n=528$, Fig. 2). There was a positive dependency between having heard or not about cervical cancer, with having heard or not about HPV ($X^2=5.267$; d.f.=1; $p=0.0217$). Yet, of the students who stated they knew what cervical cancer is and had heard of HPV, only 40.3% stated HPV has a relation to cervical cancer.

**Clinical manifestations:** In recognizing cervical cancer symptoms, 69.4% of students identified vaginal bleeding and 48.2% identified pain during sexual intercourse (Table 2). There was also a positive relationship between age and likelihood of answering a correct symptom ($F=5.18$, d.f.=1, $p=0.0232$, $r^2=0.97$%; $n=528$).

**Additional information:** Overall, 39.4% of students correctly identified the cervix from an image of the female reproductive system (Table 2). There was a strong positive relationship between age and responding correctly ($F=28.1$, d.f.=1, $p<0.0001$, $r^2=5.06$; $n=528$).

**Medical services:** We found 30.6% of students knew adolescents can go alone to the clinic for medical services (Table 2). Yet, 51% of students responded they would go alone to the clinic (Table 2). There was a strong positive relationship between age and the willingness to go alone ($F=74.8$, d.f.=1, $p<0.0001$, $r^2=12.53$; $n=522$, Fig. 3). Additionally, mean male response ($\bar{x}=0.59$) was greater than mean female response ($\bar{x}=0.45$) ($F=10.96$, d.f.=1, $p=0.0010$; $n=529$).

### TABLE 2

<table>
<thead>
<tr>
<th>Questionnaire responses and the percentage of students who gave each response.</th>
<th>San Carlos, Costa Rica. March-April 2012</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Response</strong></td>
<td><strong>Percentage of Responses (%)</strong></td>
</tr>
<tr>
<td><strong>Prevention &amp; Risk Factors</strong></td>
<td></td>
</tr>
<tr>
<td>Had heard of an educational cervical cancer campaign</td>
<td>7.7</td>
</tr>
<tr>
<td>Stated cervical cancer is preventable</td>
<td>41.5</td>
</tr>
<tr>
<td>Identified an early onset of sexual activity as a risk factor</td>
<td>30.8</td>
</tr>
<tr>
<td>Identified a high number of sexual partners as a risk factor</td>
<td>37.1</td>
</tr>
<tr>
<td>Had heard of the Papanicolaou</td>
<td>67.9</td>
</tr>
<tr>
<td><strong>Infection</strong></td>
<td></td>
</tr>
<tr>
<td>Had heard of the Human Papilloma Virus</td>
<td>75.2</td>
</tr>
<tr>
<td>Stated cervical cancer is acquired only through an infection</td>
<td>72.2</td>
</tr>
<tr>
<td>Had heard of HPV and stated it has an association with cervical cancer</td>
<td>33.9</td>
</tr>
<tr>
<td><strong>Metastasis</strong></td>
<td></td>
</tr>
<tr>
<td>Identified vaginal bleeding as a symptom</td>
<td>69.4</td>
</tr>
<tr>
<td>Identified pain during sexual intercourse as a symptom</td>
<td>48.2</td>
</tr>
<tr>
<td>Stated cervical cancer is fatal</td>
<td>63.8</td>
</tr>
<tr>
<td><strong>Additional Information</strong></td>
<td></td>
</tr>
<tr>
<td>Correctly identified the cervix</td>
<td>39.4</td>
</tr>
<tr>
<td><strong>Contributory Facts</strong></td>
<td></td>
</tr>
<tr>
<td>Stated adolescents can go to the doctor alone</td>
<td>30.6</td>
</tr>
<tr>
<td>Stated they would go to the doctor alone</td>
<td>51.0</td>
</tr>
</tbody>
</table>
The most common reasoning for unwillingness to go alone to the doctor consisted of “fear of parental punishment”, “embarrassment of sharing personal information with parents” and “fear of the doctor”, while the two least common reasons were “embarrassment with friends” and “embarrassment with boyfriend/girlfriend”. Mean female response ($\bar{x}=0.30$) was greater than male response ($\bar{x}=0.13$) for choosing “fear of the doctor” ($F=20.1$, d.f.=1, $p<0.0001; n=529$).

**DISCUSSION**

A disconnection existed between the knowledge of adolescents with various cervical cancer concepts. For example, nearly all of the students identified at least one risk factor, but less than half stated cervical cancer is preventable. Hence, the majority of students did not understand that avoiding risk factors prevents the acquisition of the disease. Additionally, only less than half of the students who knew what cervical cancer is and had heard

### TABLE 3

<table>
<thead>
<tr>
<th>Question</th>
<th>F-Value</th>
<th>df</th>
<th>n</th>
<th>P-Value</th>
<th>R² (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preventative Measures</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have you heard of the Papanicolaou (PAP)?</td>
<td>27.10</td>
<td>1</td>
<td>528</td>
<td>&lt;0.0001</td>
<td>4.88</td>
</tr>
<tr>
<td>Infection Development</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have you heard of HPV?</td>
<td>61.88</td>
<td>1</td>
<td>528</td>
<td>&lt;0.0001</td>
<td>10.49</td>
</tr>
<tr>
<td>Metastasis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Identify the symptoms of cervical cancer</td>
<td>5.18</td>
<td>1</td>
<td>528</td>
<td>=0.0232</td>
<td>0.97</td>
</tr>
<tr>
<td>Additional Information</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Identify the cervix</td>
<td>28.14</td>
<td>1</td>
<td>528</td>
<td>&lt;0.0001</td>
<td>5.06</td>
</tr>
<tr>
<td>Contributory Facts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Can an adolescent go without their parent/guardian to the clinic for medical attention?</td>
<td>32.36</td>
<td>1</td>
<td>528</td>
<td>&lt;0.0001</td>
<td>5.78</td>
</tr>
<tr>
<td>Would you go to the clinic without your parent/guardian for medical attention?</td>
<td>74.78</td>
<td>1</td>
<td>522</td>
<td>&lt;0.0001</td>
<td>12.53</td>
</tr>
</tbody>
</table>

---

Fig. 2. Relationship between student age (12-22yrs old) and response when asked, “Have you heard of the Human Papilloma Virus?” Values of “0” and “1” represent responses of “no” and “yes”, respectively. Equation is: “Heard of HPV?”=-0.2353+0.0640*Age. Concave lines are 95% confidence intervals of the estimated line. San Carlos, Costa Rica. March-April 2012.
of HPV, also stated that HPV has a relation to cervical cancer.

The questionnaire included two open-ended questions to allow for students to demonstrate their level of knowledge of cervical cancer and the Papanicolaou. While less than one-third of students stated they knew what cervical cancer is, when asked to define the disease, the majority of responses consisted of “cancer of the cervix” and possibly included information describing it as sexually transmitted or a female disease. This showed the students were lacking a depth of knowledge. Only three students were able to connect the Papanicolaou with cervical cancer and only five students mentioned prevention. This demonstrated a lack of understanding between connecting cervical cancer topics.

According to a study conducted in the United Kingdom, women living in socially-deprived areas also had a limited knowledge of cervical cancer risks and prevention strategies (Logan & Mcifatrick, 2011). Researchers of a similar study in South Africa, where cervical cancer is also the second-leading cause of death among women, stated prevention methods and materials are necessary to inform women about HPV, cervical cancer, screening and discussing the topic with children (Francis et al., 2011).

With a few principal questions, a positive correlation existed between age and knowledge of cervical cancer and medical services, specifically: identifying the cervix, identifying symptoms, hearing of the Papanicolaou, hearing of HPV and knowing adolescents can go alone to the doctor. A study in Nigeria found only 15% of participants knew of the availability of cervical cancer screening, which had a positive correlation with the level of education attainment (Nwankwo et al., 2011). The number of students in our study that knew they could go to the doctor alone, which had a positive correlation with the level of education attainment, was nearly double the amount in the Nigerian study.

As students aged, they were more likely to be willing to go alone to the doctor. With age, the students likely feel more independent. Also as they aged, the reasoning of students for not going to the clinic alone changed from “fear of parental discipline” to “embarrassment of sharing personal information with their parents”. The older the student, the more likely they were to have been sexually active and, therefore, could be associated with a reason to feel embarrassed (Picado, 2007).

Women were more likely to state they knew what cervical cancer is or have heard of the Papanicolaou. Yet, there was no significant correlation between gender and knowledge of HPV or cervical cancer risk factors. A study conducted on South African men found men lack knowledge of cervical cancer, as no

Fig. 3. Relationship between student age (12-22 years old) and response when asked if they personally would be willing to go to the clinic alone to receive medical attention. Values of “0” and “1” represent responses of “no” and “yes”, respectively. Equation is: “Would Go Alone to Clinic?” = -0.7207 + 0.0791 * Age. Concave lines are 95% confidence intervals of the estimated line. San Carlos, Costa Rica. March-April 2012.
participants mentioned the importance of HPV and only a few knew having multiple sexual partners was a risk factor (Maree et al., 2011). In contrast, about three-fourths of both Costa Rican male and female students stated they had heard of HPV, with no significant difference between genders. Maree et al. (2011) stated men need to be informed of cervical cancer as they can help protect women from risk factors and encourage screening. Although Costa Rican men had stated they had heard of HPV, few could relate the virus to cervical cancer, showing an overall lack of connection between these concepts.

Although Costa Rican females were slightly more informed of cervical cancer and the Papanicolaou, males were more likely to be willing to go alone to visit the doctor. At the same time, females were more likely to be deterred by their “fear of the doctor”. Hence, males could help support females in receiving the screening exam.

Regarding responsibility for cervical cancer prevention, in order to prevent cervical cancer, one can limit exposure to HPV infection through limiting risk factors and routinely receiving a Papanicolaou in order to prevent pre-cancerous infections from forming cancer (Feldman et al., 2011). Ideally, one would receive a HPV vaccination, but this is not covered through the CCSS, which means it is unrealistic for much of the population to receive. (A. Muñoz, personal Communication, April 16, 2012). In order to limit risk factors, education of risk factors needs to reach the population before they become sexually active, as this exposes them to HPV (American Cancer Society, 2011).

Unfortunately, the current, formal, required curriculum by the MEP does not require information on cervical cancer to be included. Fewer than one-third of students both know they can go alone and are willing to go alone. This shows few students are going alone to the clinic and, therefore, are probably less likely to receive information on sexual education from doctors. Therefore, few students receive information from educational campaigns, school or the clinic, so the majority likely isn’t receiving the key preventative measure, which is education.

Hence, the majority of the responsibility of cervical cancer prevention falls upon CCSS intervention after the onset of sexual activity and potential exposure to HPV, by providing Papanicolaou exams in order to detect any pre-cancerous or cancerous lesions. There are also potential barriers preventing women from receiving routine Papanicolaou, as only 44.8% of Costa Rican women are yearly screened (Aguado et al., 2010). A study conducted in the United States found distrust in the healthcare system was negatively correlated with utilization of cervical cancer screening (Tse-Chuan, Matthews, & Hillemeier, 2011). Although we did not measure health care system distrust, nearly half of students with reasoning for not going alone to the clinic chose, “fear of the doctor”. Hence, this could prevent utilizing the accessible screening exam. Therefore, it is even more crucial to educate the population on risk factors.

As regards intervention strategies, this study showed students were lacking information not only on cervical cancer. High school students would benefit from a specific sexual education course. Otherwise, cervical cancer could be added to the already required curriculum pertaining to sexually transmitted diseases. Currently health discussions are given 2-3 times a year by the CCSS to the student population, but this is dependent on funding. Hence, we stress the importance of keeping these health discussions a priority of the community.

Less than half of the students who completed the questionnaires were willing to go to the doctor alone, nearly one quarter of these students because of “embarrassment of sharing personal information with their parents”. Hence, it is also possible that students accompanied by their parents would avoid correctly filling out the risk classification sheet in the case they are fearful of the response of their parents. We think it would be ideal for the CCSS to offer yearly physicals for students at
the high schools so students would feel more comfortable going alone to visit the doctor.

The limitations or obstacles we faced included a low return rate of parental consent forms, from forgetfulness or unwillingness of students and/or parents. When there were only a few students of a classroom with a signed permission slip, it was more likely they would choose not to participate. In the Colegio Técnico de Venecia, the principal distributed a consent form, containing incorrect information, to students before we went to the high school. Therefore, students with these consent forms could not participate either.

In a few classes, teachers would discuss cervical cancer after their students asked questions about the study. Students also discussed the questions between themselves, during and after taking the questionnaire. This could distort the data, as the discussions were only prompted by our presence.

With regard to future research, this study created new questions concerning the barriers of cervical cancer screening. As in the one EBAIS we visited, the male doctor had a significantly lower number of Papanicolaou exams administered in 2011 than the female doctor; it would be important to analyze the mean number of Paps administered between male and female providers. Alongside, it would be beneficial to compare women’s comfort levels in receiving the exam by doctors of each gender.

Three formal cervical cancer education methods exist for adolescents in the San Carlos region: Semana de Salud once a year, discussions by medical doctors in the high schools and visits with the doctor. The trend that males were more likely to hear of an educational cervical cancer campaign in their community lacks an explanation and would be a source for future study. Additionally, it is important to know which of these education types are most effective, in order to ensure teenagers are receiving the information in an appropriate manner.

ACKNOWLEDGMENTS

We would like to thank our advisors, Diana and Freddy Ulate, who helped us design and carry out a realistic study that is beneficial to Costa Rica. Additionally, we would like to acknowledge the support of ACM staff, especially Michael McCoy and Chris Vaughan, who helped us along every step of the research process. Thanks also go to the student participants, as without their willingness and initiative we would not have a study. The warm welcome and above and beyond patience of the superintendent, directors, principals, teachers and secretaries within the Ministry of Public Education were utterly appreciated.

RESUMEN

Conocimiento sobre cáncer de cérvix entre colegiales de San Carlos, Costa Rica. En Costa Rica, el cáncer de cérvix es el segundo tipo de cáncer femenino más común, a pesar de la accesibilidad del Papanicolaou gracias al sistema de salud universal. No se ha evaluado el nivel de conocimiento de la población adolescente sobre el cáncer de cérvix, aunque este grupo es el más expuesto a los factores de riesgo. Este estudio investigó cuánto sabe el adolescente sobre métodos de prevención, riesgo de infección, manifestaciones clínicas y servicios médicos. En total, 533 estudiantes de séptimo a doceavo año de cinco colegios públicos de la zona rural de San Carlos participaron en esta investigación durante marzo y abril 2012. Se observó que los estudiantes carecen de información, ya que solamente el 30.8% sabían qué es el cáncer de cérvix. Nuestros resultados indican que es importante incluir el cáncer de cérvix en los programas de educación sexual del sistema de educación pública de Costa Rica, para educar a la población en riesgo acerca de los métodos de prevención, desarrollo de la infección y servicios médicos.

Palabras clave: adolescentes, cáncer de cérvix, Costa Rica, educación sexual, Papanicolaou.
REFERENCES


Effects of different agricultural systems on soil quality in Northern Limón province, Costa Rica

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Abstract: Conversion of native rainforest ecosystems in Limón Province of Costa Rica to banana and pineapple monoculture has led to reductions in biodiversity and soil quality. Agroforestry management of cacao (Theobroma cacao) is an alternative system that may maintain the agricultural livelihood of the region while more closely mimicking native ecosystems. This study compared physical, biological and chemical soil quality indicators of a cacao plantation under organic agroforestry management with banana, pineapple, and pasture systems; a native forest nearby served as a control. For bulk density and earthworm analysis, 18 samples were collected between March and April 2012 from each ecosystem paired with 18 samples from the cacao. Cacao had a lower bulk density than banana and pineapple monocultures, but greater than the forest (p<0.05). Cacao also hosted a greater number and mass of earthworms than banana and pineapple (p<0.05), but similar to forest and pasture. For soil chemical characteristics, three composite samples were collected in March 2012 from each agroecosystem paired with three samples from the cacao plantation. Forest and pineapple ecosystems had the lowest pH, cation exchange capacity, and exchangeable nutrient cations, while cacao had the greatest (p<0.05). Total nutrient levels of P and N were slightly greater in banana, pineapple and pasture than in cacao; probably related to addition of chemical fertilizer and manure from cattle grazing. Forest and cacao also had greater %C, than other ecosystems, which is directly related to soil organic matter content (p<0.0001). Overall, cacao had more favorable physical, biological and chemical soil characteristics than banana and pineapple monocultures, while trends were less conclusive compared to the pastureland. While organic cacao was inferior to native forest in some soil characteristics such as bulk density and organic carbon, its soil quality did best mimic that of the native forest. This supports the organic cultivation of cacao as a desirable alternative to banana and pineapple monoculture. Rev. Biol. Trop. 62 (3): 887-897. Epub 2014 September 01.

Key words: agroforestry, banana (Musa acuminata), cacao (Theobroma cacao), Costa Rica, organic, pineapple (Ananus comosus), pasture, soil quality.

In the Caribbean coastal plain of Costa Rica, conversion of native rainforest ecosystems to monocrop agriculture production has led to habitat loss and biodiversity reductions (Vaughan, Ramirez, Herrera, & Guries, 2007). Agroforestry systems, consisting of shade trees integrated among primary crops, can more closely mimic the floral and faunal biodiversity of native forests. Past research has shown that after many years of agroforestry management, fields can attain levels of soil quality equal to those of an adjacent native forest system (Isaac, Gordon, Thevathasan, Oppong, & Quashie-Sam, 2005).

One crop that is commonly grown under agroforestry management is cacao (Theobroma cacao). Previous studies indicated that, in comparison to monocrop systems, cacao cultivation has larger nutrient inputs, reduced nutrient losses, and better soil physical properties such as penetrability and bulk density (Young, 1989; Isaac et al., 2005; McNeely & Schroth, 2006; Vallejo, Roldan, & Dick, 2010; Tully & Lawrence, 2011).
Soil health is very important to successful agriculture. A high quality soil provides an environment for optimum root growth that enhances crop health and productivity (Baldwin, 2006). Beyond merely enhancing the growth of one desired crop, a balanced, healthy soil can sustain the productivity of other animals and plants, support human health, and improve the quality of air and water (Doran, Sarrantonio, & Liebig, 1996; Bonanomi et al., 2011). Because healthy soil can benefit whole ecosystems, analyzing the soil quality of a certain system of agriculture can provide a strong case for or against its environmental sustainability.

Soil quality is a complex entity that cannot be measured directly. Instead, physical, chemical and biological indicators must be considered. Physical factors such as bulk density give an indication of the quality of soil for adequate root growth and movement of water and air (USDA, 1999). In addition, macroinvertebrate and microbial communities are essential factors to consider in the maintenance of soil fertility, because they play crucial roles in influencing physical and chemical properties of the soil (Sánchez-de Leon, de Melo, Soto, Johnson-Maynard, & Lugo-Perez, 2006). It is also very important, especially in tropical ecosystems, to maintain high levels of soil organic matter, because this is the primary source of energy, nitrogen and soil structure for soil and plant ecosystems (Sánchez-de Leon et al., 2006). Compared to monocrop systems, agroforestry has been shown to have more abundant and diverse microfauna, and greater levels of soil organic matter (Sánchez-de Leon et al., 2006).

One can also note differences in the soil quality of farms under organic vs. conventional management. The use of chemical fertilizers and pesticides on “conventionally” managed farms can lead to polluted runoff that has detrimental effects on the organisms in the wider ecosystem (Castillo et al., 2006). These chemicals can also affect soil health.

The purpose of this study was to compare physical, biological, and chemical indicators of soil quality on an organic cacao farm under agroforestry management with conventional banana and pineapple monocultures, as well as a neighboring pasture and fragment of primary rainforest. I also compared the soil quality of organic and non-organic cacao cultivation. By adding to the body of research surrounding this topic, I aimed to strengthen the case for cacao agroforestry as an alternative agriculture method that can mitigate the negative effects of deforestation while still providing a livelihood for area farmers.

MATERIALS AND METHODS

Study area: The study area is located in Pueblo Nuevo de Villa Franca de Guácimo, in Limón Province, Costa Rica (10º20’ N - 83º20’ W). This area receives a mean annual rainfall of 6000mm with a mean air temperature of 26°C and is located 40m above sea level, in the Premontane Wet Forest Life Zone (Holdridge, 1964; Vaughan et al., 2007). Major soil types (sub-group/great group/suborder/order) on this farm and surrounding areas include typic tropoquept, aeric tropoquept, aquic eutropept, aquic dystrandept, and oxic dystrandept (Obando, 1982).

The primary study site is a 110ha cacao plantation that has been under organic management since 2002. This land was originally primary rainforest, but was managed as livestock pasture for many years before its conversion to cacao agriculture in the 1980s. The primary tree crop, Theobroma cacao, is maintained under shade trees Eucalyptus deglupta, Cocos nucifera, Leucaena leucocephala, and Musa acuminata (R. Wieme, unpublished). Organic fertilizer made of composted coffee husks is applied biannually, and since 2011 an organic herbicide has been applied for weed control; no chemical fertilizers or pesticides have been used in the prior 13yrs. A subterranean system of drainage tubes was installed in 1997 to help maintain adequate soil moisture content (G. Herrera, pers. comm., wesgeova@yahoo.com). There is a strip of cacao farmland bordering the adjacent banana plantation on which the cacao is officially classified as conventional.
A large banana (*Musa acuminata*) monoculture borders the cacao plantation on the west and is maintained with regular applications of chemical fertilizers, insecticides, and fungicides. Pasture for cattle grazing borders the north side of the cacao plantation. These pastures consist mainly of African star grass (*Cynodon nlemfuensis*) with some large isolated trees like *Coussapoa villosa*, *Ocotea sinuata*, *Erythrina poeppigiana* and *Hura crepitans* and are not chemically treated (Vaughan et al., 2007). A fragment of native primary forest is located approximately 2 km northeast of the cacao plantation.

**Selection of sampling sites:** I selected sampling sites on similar soil types located on roughly the same topographical features, according to an available soil types map (Obando, 1982). To compare banana and pasture with cacao, I selected six paired plots along the cacao plantation with each plot located approximately 15 m perpendicular to the border between cultivation types. I also selected six study sites in the pineapple monoculture and three control sites in the natural primary forest. For analysis, these sites were paired with six cacao sites to minimize variation due to sampling time; samples for bulk density and earthworm abundance from paired sites were collected on the same day.

**On-site analysis of bulk density:** At each sampling site described above, I extracted three core samples of known volume to approximately 10 cm depth at each sampling site; I weighed these samples to determine their fresh weight. A subsample of each soil core was weighed, dried in a microwave oven until mass no longer decreased, and then weighed again to determine dry weight. To calculate the oven-dried weight of the full sample, I used the formula \( W_d = \frac{W_{f} x W_{dsub}}{W_{fsub}} \), where \( W_d \) = oven dried weight of full sample, \( W_f \) = fresh weight of full sample, and \( W_{dsub} \) and \( W_{fsub} \) are the dry and fresh weights of the subsample. I then calculated bulk density using the formula \( (W_d/V) \), where \( V \) = soil core volume.

**On-site analysis of earthworm abundance:** Using a 25 x 25 cm quadrant frame, I excavated the soil at each paired sampling site to a depth of 20 cm and searched by hand for earthworms (Gregory, Shea, & Bakko, 2005; Sánchez-de Leon et al., 2006). I obtained three replicates at each sampling site and determined the total number and mass of earthworms. Because earthworm abundance varied greatly depending on weather, earthworms from paired sites were collected on the same day; all samples were collected between March 8 and April 19, 2012.

**Laboratory analysis of soil samples:** For chemical laboratory analysis, I took samples at three of the six paired sampling sites for each agricultural ecosystem on March 27, 2012. At each site, I combined five cores, 10 cm deep by 4 cm wide. Samples were sent to the Centro de Investigaciones Agronómicas at the University of Costa Rica, San Pedro de Montes de Oca for laboratory analysis. Chemical laboratory analysis included pH, acidity, cation exchange capacity, concentrations of macro- and micro-nutrients, %C and %N.

I used Statgraphics Centurion XVI software (Statpoint Technologies, Inc., 2012) to analyze each soil quality indicator separately for significant differences between crop types. For analysis of the forest ecosystem, I used one-way ANOVA tests. The other ecosystems were compared to their corresponding paired sites in the cacao using paired comparisons two-way parametric ANOVA tests, in order to account for some of the inherent microscale variation in soil characteristics. All ANOVAs were performed after testing for normality and homogeneity of variance (Sokal & Rohlf, 2012). Unplanned comparisons of means were...
estimated with least significant, Tukey or Scheffe confidence intervals.

**RESULTS**

**Bulk density:** Among all ecosystems, mean soil bulk density was greatest in pineapple (\(\bar{x}=0.79g/cm^3\)) and lowest in forest (\(\bar{x}=0.48g/cm^3\)) (F=13.1; df=7, 127; p≤0.0001, Fig. 1). Forest soil bulk density was less than any other crop, including its paired cacao site (0.66g/cm³) (p<0.05, Fig. 1). Banana (\(\bar{x}=0.69g/cm^3\)) had greater bulk density than its corresponding paired cacao sites (\(\bar{x}=0.61g/cm^3\)) (F=19.92; df=1, 24; p=0.0002; Fig. 1). Pineapple (\(\bar{x}=0.79g/cm^3\)) also had greater bulk density than corresponding paired cacao sites (\(\bar{x}=0.66g/cm^3\)) (F=28.17; df=1, 24; p≤0.0001; Fig. 1). The pasture system (\(\bar{x}=0.60g/cm^3\)) did not differ significantly from its paired cacao site (\(\bar{x}=0.61g/cm^3\)) (F=1.73; df=1, 24; p=0.20, Fig. 1).

**Earthworm abundance:** Mean number of earthworms did not differ significantly between forest (\(\bar{x}=13.9\)) and cacao (\(\bar{x}=15.1\)) (F=0.16; df=1, 16; p=0.69; Fig. 2). There was also no significant difference in earthworm mass between forest (\(\bar{x}=2.93g\)) and cacao (\(\bar{x}=3.55g\)) (F=0.76; df=1, 16; p=0.3965). There were fewer earthworms in the banana plantation (\(\bar{x}=1.8\)) than in its corresponding paired sites of cacao (\(\bar{x}=8.6\)) (F=38.86; df=1, 24; p<0.0001; Fig. 2). There was also a lower earthworm mass in the banana (\(\bar{x}=0.39g\)) than corresponding cacao sites (\(\bar{x}=1.73g\)) (F=7.36; df=1, 16; p=0.0153). In the pineapple plantation, there was a lower number (\(\bar{x}=1.5\)) and mass (\(\bar{x}=0.24g\)) of earthworms than in the corresponding paired sites in the cacao (number: \(\bar{x}=8.0\); mass: \(\bar{x}=1.86g\)) (number: F=48.03; mass: F=38.86; df=1, 24; p<0.0001).

![Fig. 1. Mean bulk density by ecosystem. Adjacent bars indicate mean bulk density from paired sampling sites. Error bars represent 95% LSD confidence interval. a: Bulk density was significantly different between forest and cacao (p<0.0001). One-way ANOVA, Fisher’s LSD. b: Bulk density differed significantly (p<0.0001). Two-way paired-comparison ANOVA, Fisher’s LSD. c: Bulk density was not significantly different (p>0.05). Two-way paired-comparison ANOVA, Fisher’s LSD. Pueblo Nuevo de Guácimo, Limón Province, Costa Rica. March-April 2012.](image-url)
df=1, 24; p≤0.0001; mass: F=26.19; df=1, 20; p=0.0001) (Fig. 2). The pasture ecosystem had a greater number of earthworms (x̄=11.9) than the corresponding cacao paired sites (x̄=6.6) (F=7.91; df=1, 24; p=0.0096; Fig. 2). However, pasture did not have a significantly different earthworm mass (x̄=2.30g) than corresponding paired cacao sites (x̄=1.80g) (F=0.61; df=1, 16; p=0.45).

**Physicochemical soil characteristics:**
Mean pH was lower in the forest (x̄=4.3) than the cacao (x̄=5.8) (p<0.01; Table 1). Pineapple (x̄=4.6) also had a lower pH than its corresponding cacao paired sites (x̄=5.8) (F=61.7; df=1, 2; p=0.0158; Table 1). In addition, pasture sites had lower pH (x̄=5.2) than corresponding cacao paired sites (x̄=5.6) (F=24.14; df=1, 2; p=0.0390; Table 1). Banana pH (x̄=5.7) did not differ significantly from its corresponding paired sites in the cacao (x̄=6.0) (Table 1).

Cation exchange capacity (CEC) was greater in the cacao (x̄=22.55cmol+/L) than the forest (x̄=10.45cmol+/L) (p<0.05; Table 1). Cacao CEC was also greater (x̄=22.55cmol+/L) than in corresponding paired sites of pineapple (x̄=16.74cmol+/L) (F=53.9; df=1, 2; p=0.0087; Table 1). Cacao also had a greater CEC (x̄=21.16cmol+/L) than corresponding paired sites in pasture (x̄=15.93 cmol+/L) (Table 1).

**Exchangeable nutrient cations:** Forest had less mean concentration of Ca²⁺ (x̄=3.90cmol+/L) than cacao (x̄=17.10cmol+/L) (p<0.01; Table 1). Pineapple also had less Ca²⁺...
(\(\bar{x}=9.80\text{ cmol}^+\text{/L}\)) than corresponding paired sites in the cacao (\(\bar{x}=17.10\text{ cmol}^+\text{/L}\)) (\(F=53.6; \) df=1, 2; \(p=0.0182\); Table 1). Similarly, Ca\(^{2+}\) was lower in pasture (\(\bar{x}=11.72\text{ cmol}^+\text{/L}\)) than cacao (\(\bar{x}=16.13\text{ cmol}^+\text{/L}\)) (\(F=121; \) df=1, 2; \(p=0.0081\); Table 1). Banana, however, did not have a significantly different level of Ca\(^{2+}\) (\(\bar{x}=10.33\text{ cmol}^+\text{/L}\)) than corresponding cacao paired sites (\(\bar{x}=11.85\text{ cmol}^+\text{/L}\)) (Table 1).

Forest (\(\bar{x}=2.19\text{ cmol}^+\text{/L}\)) had less Mg\(^{2+}\) than cacao (\(\bar{x}=4.81\text{ cmol}^+\text{/L}\)) (\(p<0.05\); Table 1). Mean concentrations of Mg\(^{2+}\) in the other ecosystems varied from 3.39cmol\(^+\)\text{/L} to 4.81cmol\(^+\)\text{/L}, and none differed significantly from corresponding paired sites in the cacao (Table 1).

Reflecting the trends in Mg\(^{2+}\), the only significant difference in K\(^{+}\) concentration was between forest (\(\bar{x}=0.16\text{ cmol}^+\text{/L}\)) and cacao (\(\bar{x}=0.52\text{ cmol}^+\text{/L}\)) (\(p<0.05\); Table 1). While no other ecosystems differed significantly from corresponding cacao paired sites, K\(^{+}\) concentration in banana (\(\bar{x}=0.78\text{ cmol}^+\text{/L}\)) tended to be greater than that in cacao (\(\bar{x}\)
=0.52 cmol+/L). The K+ concentration in pasture (\(\bar{x}=0.28\) cmol+/l) tended to be less than that in cacao (\(\bar{x}=0.50\) cmol+/L) (Table 1).

**Total nutrients:** Mean phosphorus levels were significantly greater in forest (\(\bar{x}=25.6\) mg/L) than cacao (\(\bar{x}=12.3\) mg/L) (Table 2). In the other ecosystems, there was great variation in P levels among sampling stations, even within the same ecosystem. Although there were no significant differences between ecosystems, the organic cacao consistently had a lower mean P level than its corresponding paired sites in banana, pineapple, and pastureland (Table 2).

Mean percentage of N did not differ significantly between forest (\(\bar{x}=0.49\)%) and cacao (\(\bar{x}=0.37\)%) (p>0.05; Table 2). Other ecosystems showed very similar mean %N values which did not differ significantly from corresponding paired sites in the cacao; values ranged from 0.37% to 0.47% with the exception of pineapple, which had a much lower %N (\(\bar{x}=0.15\)%) than its paired cacao sites (\(\bar{x}=0.37\)%) (\(F=43.6; df=1, 2; p=0.0222\); Table 2).

**Soil organic carbon:** Mean percentage of organic carbon (%C) did not differ significantly between the forest ecosystem (\(\bar{x}=5.59\)%) and cacao (\(\bar{x}=3.85\)%) (p>0.05; Table 2). However, mean percentage of C was lower in the pineapple (\(\bar{x}=1.34\)%) than corresponding paired sites in the cacao (\(\bar{x}=3.85\)%) (\(F=82.6; df=1, 2; p=0.0119\); Table 2). Percentage of C in banana and pasture did not differ significantly from that of corresponding paired sites in organic cacao, ranging from 3.88% to 4.39% (Table 2).

All results of the above comparisons (Tables 1, 2) for “conventional cacao” plots did not differ significantly from those of their paired sites in the organic cacao.

**DISCUSSION**

Soil bulk density is an indication of soil physical structure. A lower bulk density facilitates unrestricted root growth, oxygen and water flow through the soil (USDA, 1999). The lower bulk density in the forest and cacao indicated that the soil structure in these systems was more favorable for plant growth than that of the banana and pineapple monocultures. This could be associated with the greater abundance of earthworms and more complex root structure in these agriculture systems. Abundant trees in these systems also add organic material to the soil through litterfall which may contribute to lesser soil compaction. Ten years of organic compost addition to the cacao soil probably was the greatest factor in improving its soil bulk density. In contrast, all natural vegetation is removed from the banana and pineapple plantations for “monocrop” cultivation, leaving the soil exposed to the elements. Intensive cropping, trampling of soil by workers, lack of organic material addition, and pounding of rain can compact the soil, leading to an undesirably high bulk density (Hajabbasi, Jalalian, & Karimzadeh, 1997).

The soil in the cacao had a greater bulk density than that in the forest, suggesting that while preferable to banana and pineapple monoculture, agroforestry management did not attain a soil structure as favorable as the natural forest. A study of livestock pasture found a similar relationship between cacao and forest bulk density, showing that an agroforestry system had intermediate levels of soil compaction and bulk density between a conventional monocropped pasture and natural forest (Vallejo et al., 2010). While I didn’t find significant differences between cacao and pasture, the cacao plantation was previously managed as pasture before its conversion 30 years ago; previous research has found that, over time, soil under agroforestry management can continue to decrease in bulk density (Isaac et al., 2005).

Earthworm abundance was greater in the cacao than in the monoculture banana and pineapple systems, and was similar between the cacao and forest. This suggests that agroforestry management of cacao better mimics the macroinvertebrate population of a natural rainforest. This could be related to the presence of shade trees in the cacao and forest, which create a favorable environment for earthworms.
A study of earthworms in coffee plantations in Turrialba, Costa Rica found that earthworm density and fresh weight were greater in plantations under shade vs sun, and organic vs. conventional management (Sánchez-de Leon et al., 2006).

Earthworms usually have positive effects on soil quality: as they feed and burrow through the soil, they aid in decomposition of organic matter, nutrient cycling, microbial activity, soil porosity and bulk density (Fragoso et al., 1997; Brown et al., 1999). However, they can also negatively affect agricultural production by competing with plants for water and nutrients (Brown et al., 1999). The specific benefits or drawbacks to earthworm presence depend on the type of agricultural system and the species of earthworms present (Fragoso et al., 1997, Brown et al., 1999). Exotic earthworms can replace native species, reducing the productivity of the ecosystem (Fragoso et al., 1997). Exotics can tolerate a wider range of soil conditions, while native earthworms usually can’t survive in disturbed ecosystems like banana or pineapple monocultures (Lee, 1983). Case studies have shown that in tropical ecosystems native earthworm species are more likely to be maintained when there are many trees, chemicals are not used, and organic inputs are dominant in the system (Fragoso et al., 1997). It was not within the scope of this study to classify the earthworms found in the different agricultural systems into species or functional groups, but future studies might find that the species composition of earthworms differs depending on the agricultural system.

Regarding physicochemical soil characteristics, the more neutral pH that I found in cacao as compared to the banana and pineapple monocultures is consistent with previous research showing greater pH in agroforestry than monocultures (Sharma et al., 2009). In tropical forest and agroforestry systems, the deposition of bases over many years of tree growth can increase the pH of the soil, buffering the effects of nutrient leaching (Young, 1989; Nair, Buresh, Mugendi, & Latt, 1999; Sharma et al., 2009). The pH in the native forest was the lowest of all ecosystems studied. Forests in the humid tropics of Latin America have a greater percentage of acid soils (82%) compared to those in Africa (56%) or Asia (38%) (Sánchez, 1987).

Cation exchange capacity (CEC) indicates the nutrient retention capability of a soil by measuring the quantity of available negative charges to which nutrient cations such as K⁺, Ca²⁺, and Mg²⁺ can bind. A greater CEC provides a potential bank of nutrients to replace those taken up by plants or leached by rainwater (Camberato, 2001). Soil with greater quantities of clay and organic matter tends to have greater CEC (Camberato, 2001). In my study, CEC was lowest in the native forest, even though it had a greater amount of organic carbon. This is probably associated with the low pH in the forest; more acidic soils have fewer negative charges, and many of the binding sites are occupied by H⁺ and Al³⁺ cations (Al³⁺ is present in cation form at pH lower than 5.0) (Lines-Kelly 1993). This trend of increasing CEC with greater pH carried overall among all ecosystems. Of the agricultural systems, CEC was lowest in the banana and pineapple monocultures, intermediate in the pasture, and greatest in the cacao. This is consistent with the results of a past study which found significantly greater CEC under an agroforestry system than pastoral land, and consequently greater CEC under pastoral than arable land (Sharma et al., 2009).

Regarding exchangeable nutrient cations, such cations as Ca²⁺, Mg²⁺, and K⁺ occupy the negative binding sites in the soil indicated by CEC. As with CEC, native forest had the lowest levels of Ca²⁺, Mg²⁺, and K⁺ of all ecosystems. This contradicts past research which suggests that decomposition of litterfall and neutral soil reactions lead to greater nutrient cation concentrations in tree-based systems (Lal, 1989; Sharma et al., 2009). Greater nutrient cation concentrations in the agricultural systems may be attributed to the application of fertilizer, whether organic (such as the compost used in the cacao) or chemical (for example, it appeared that calcium carbonate had recently
been applied to the banana plantation when soil samples were taken). The cacao had significantly more Ca\(^{2+}\) than pasture or pineapple sites, and while I did not find a significant difference between banana and cacao, this may have been related to the recent application of calcium carbonate in the banana. Differences among agricultural systems in Mg\(^{2+}\) and K\(^{+}\) were not as great, although previous research has found that agroforestry systems have greater amounts of these nutrients than pastoral or arable land (Sharma et al., 2009).

Regarding total nutrients, cacao tended to have lower levels of phosphorus (P) than its corresponding paired sites in the banana and pineapple although these differences were not significant. P was also significantly greater in the forest than the cacao. Nitrogen (N) percentage did not differ as greatly among ecosystems as P, but was significantly lower in the pineapple than the cacao. These results contradict previous studies which have shown that levels of N, P, and potassium were all greater under tree cover than in open field monocultures (Aggarwal, 1980; Radwanski & Wickens, 1981; Young, 1989). The greater levels of P in banana and pineapple may be explained by chemical fertilizer application which artificially increases nutrient levels. Future studies analyzing nutrient content of rainwater runoff would help determine if nutrients such as N and P are being applied in excess, an economically ineffective and environmentally damaging practice.

Cacao also had slightly lower P and %N than pasture. Sharma et al. (2009) attributed higher levels of N and P in pastoral land than an agroforestry system to nutrients added by manure from grazing cattle.

Regarding soil organic carbon, the percentage of organic carbon in the soil is directly related to the soil organic matter (SOM), and can therefore be used to model SOM levels. While not significantly different, the forest did have greater %C than the cacao and the greatest %C overall. Although the only ecosystem with a significantly lower %C than organic cacao was pineapple; banana had slightly lower %C as well. Previous research has also found greater SOM in native forest and agroforestry systems than monocropping, which has been attributed to greater root biomass and litterfall (Szott, Fernandes, & Sánchez, 1991; Madhumitha, Singh, & Khan, 1997; Sharma et al., 2009). The pasture had a slightly greater %C than its paired sites in the cacao, but not significantly so. Past studies comparing pasture and agroforestry systems have had conflicting results: some found that pasture has the capacity to sequester more carbon, while others found more carbon in agroforestry systems (Lantz, Lal, & Kimble, 2001; Sharma et al., 2009).

This study indicates that organic agroforestry management of cacao results in soil with greater overall quality for plant and animal health than banana and pineapple monocultures. Shade trees add organic material through litterfall and provide a haven for wild animals, which in turn add their own nutrients and organic matter to the soil. The lack of chemical additives allows native macroinvertebrates such as earthworms to flourish, which also improves bulk density and nutrient content. When agriculture is managed to mimic a native rainforest ecosystem, the results of this study showed that many factors work together in a complicated net of interactions to maintain soil fertility.

Banana and pineapple monocultures, in contrast, maintain high levels of productivity only with the addition of chemical fertilizers and pesticides. As this study shows, soil quality is reduced; it is unlikely that this soil will support such aggressive management for many years without a drastic decline in productivity.

Because many residents of Limón Province rely on agriculture as their livelihood, it is essential to maintain the fertility of the soil so that it can support the healthy crop growth in future generations. Banana and pineapple growers may try to mimic the agroforestry approach of cacao production, imitating as closely as possible the native ecosystem. This might involve allowing ground cover to prevent soil compaction; finding organic alternatives to fertilizers and pesticides; or even developing a
more integrated agroforestry approach like that of the cacao plantation. There are examples of the successful cultivation of banana and pineapple alongside other crop plants (such as cacao) in agroforestry systems (Khaleque & Gold, 1993; Jose, 2009). Agroforestry management of not just cacao but banana and pineapple as well may indeed be the best way to maintain soil quality in the region.

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RESUMEN

Efectos de sistemas agrícolas diferentes sobre calidad de suelos en el norte de la provincia de Limón, Costa Rica. Muchos de los ecosistemas boscosos naturales de la provincia de Limón, Costa Rica, se han convertido en monocultivos de banano y piña, lo que ha reducido la biodiversidad y la calidad de los suelos. El manejo agroforestal del cacao (Theobroma cacao) es un sistema alternativo, que puede generar ingresos para los agricultores de la región mientras imita mejor los ecosistemas nativos. En esta investigación se compararon los indicadores físicos, químicos y biológicos de la calidad del suelo en un cacao en monocultivo y agroforestal, una plantación banana, una piñera, un potrero y un bosque natural. En general, el cacao en el cacao tuvo características físicas, biológicas y químicas mejores que los monocultivos de banano y piña, mientras las tendencias en comparación con el potrero fueron menos marcadas. Aunque algunas características del suelo en el cacao orgánico, como densidad y cantidad de carbono, fueron inferiores al bosque nativo, la calidad del suelo en el cacaotal imitó mejor la del bosque nativo. Los resultados de esta investigación apoyan el cultivo de cacao como una alternativa deseable al monocultivo de banano y piña.

Palabras clave: banano (Musa acuminata), cacao (Theobroma cacao), calidad del suelo, Costa Rica, manejo agroforestal, cultivo orgánico, piña (Ananas comosus), potrero.

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Use of *Trichoderma* fungi in spray solutions to reduce *Moniliophthora roreri* infection of *Theobroma cacao* fruits in Northeastern Costa Rica

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Abstract: Cacao (*Theobroma cacao*) is an important cash crop in tropical climates such as that of Latin America. Over the past several decades, the infection of cultivated cacao by *Moniliophthora roreri*, known commonly as “monilia”, has significantly hindered cacao production in Latin America. Studies have proposed the use of *Trichoderma* sp. fungi in biocontrol treatments to prevent and reduce monilia infection, yet tests of *Trichoderma*-containing spray treatments on cacao agroforests have produced mixed results. Researchers and agricultural workers have suggested that addition of soil, fly ash, or other carbon sources to a *Trichoderma* spray may improve its efficacy in fighting monilia. To test these suggestions, we designed a series of spray mixtures including *Trichoderma* cultures, soil, and all necessary controls. We applied the spray mixtures to 80 cacao trees (20 trees for each of four resistant-selected clones to monilia) at the FINMAC organic cacao plantation in Pueblo Nuevo de Guacimo, Limón Province, in northeastern Costa Rica in March-April 2013. Five treatments were applied (control, water, water plus sterilized soil, water plus *Trichoderma*, and water plus sterilized soil plus *Trichoderma*). Each treatment was applied to four trees of each clone. We monitored the incidence of monilia infection under each spray treatment over the course of 35d. We found that spraying entire cacao trees two times with a mixture containing *Trichoderma* and sterilized soil significantly reduced the incidence of monilia infection by 11% (p<0.05) in only 35d, as compared to the control. This reduction in loss of cacao pods translates into an increase of plantation mean productivity of 1 500kg dried beans/ha by 198kg/ha up to 1 698kg/ha or by a total increase over the whole 110ha plantation by 21 780kg. We propose that using such an antifungal spray over the whole course of a crop cycle (120 days) would decrease infection incidence even more. Application of this fungal control measure has the potential of revitalizing the production of cacao in the region. Rev. Biol. Trop. 62 (3): 899-907. Epub 2014 September 01.

Key words: *Theobroma cacao*, biological control, cacao, Costa Rica, monilia, organic fungicide, *Trichoderma*.

Cultivation of the cacao tree, *Theobroma cacao* (L.), is very important to the economies and ecosystems of Latin American countries such as Costa Rica. Cacao seeds, the primary ingredient in chocolate, are a valuable cash crop which historically has comprised a large amount of Latin American agricultural exports (Bamba & Reed, 2004). In addition, cacao agroforest systems are thought to provide habitat and migration corridors between forest fragments for such diverse species as sloths (Vaughan, Ramirez, Herrera, & Guries, 2007; Ramirez, Vaughan, Herrera, & Guries, 2011), howler monkeys (Muñoz, Estrada, Naranjo, & Ochoa, 2006), numerous birds (Reitsma, Parrish, & McLarney, 2001), foraging ants (Roth, Perfecto, & Rathcke, 1994), terrestrial amphibians and reptiles (Whitfield et al., 2007) and dung beetles (Harvey, González, & Somarriba, 2006). Yet the Latin American cacao industry today is struggling with persistent crop disease.

The infection of cacao fruits throughout Central and South America by the fungus *Moniliophthora roreri*, known commonly
as “monilia”, has decimated much of Latin American cacao production over the past four decades (Hoopen, Rees, Aisa, Stirrup, & Krauss, 2003; Aime & Phillips-Mora, 2005; Phillips-Mora & Wilkinson, 2007). Monilia infection is extremely concerning to chocolate producers. Throughout Latin America, monilia infection claims some 30 000MT of potential cacao production annually (Bowers, Bailey, Hebbing, Sanogo, & Lumsden, 2001). Additionally, loss of this agroforestry crop as an economic option has aided the recent expansion of banana and pineapple monocultures, very destructive ecologically, as well as socially.

Recent studies demonstrated the potential of the fungus, *Trichoderma* sp., which thrives in the soil of cacao agrosystems and similar environments (Mpika, Kebe, & N’Guessan, 2011), as a natural antagonist to *M. roreri* (Stefanova, Leiva, Larrinaga, & Coronado, 1999; Benítez, Rincón, Limón, & Codón, 2004, Nalimova, 2007). Some plantations, including FINMAC, have proposed a passive management strategy where diseased pods are cut from trees and buried under the leaf litter near the topsoil under the assumption that the soil population of *Trichoderma* will kill off the monilia spores as described in Krauss & Soberanis (2002). Yet the current rate of loss attributed to monilia at FINMAC (25-30% pod loss/ yr) indicates that simply burying the pods and using semi-resistant on-farm-produced clones is not enough to combat infection effectively.

Previous research also suggested the possibility of spraying cacao pods, branches, and/or trunks with *Trichoderma* cultures as a preventative management strategy to control the spread of monilia. However, *Trichoderma* normally thrives in the soil, meaning that the fruits and branches of a cacao tree do not provide ideal growth conditions or nutrients. To compensate for this environmental mismatch in agricultural applications, some studies have proposed and tested (Krauss & Soberanis, 2002) the addition of nutrient sources when applying a *Trichoderma* spray.

The objectives of this study were to test the temporal impact of *Trichoderma* fungal spray: a) on reduction of monilia incidence and degree of infection, b) with and without addition of sterilized soil as a fungal nutrient or adherent, and c) on four farm-produced clonal cacao varieties.

**MATERIALS AND METHODS**

**Study site:** The study was conducted on the organic cacao (*Theobroma cacao*) plantation at FINMAC, in Pueblo Nuevo de Villa Franca de Guacimo, Limón Province, Costa Rica (10°20” N - 83°20” W). The FINMAC plantation sits at 40m above sea level (Wieme, 2011) and is located within the Premontane Wet Forest Life Zone (Holdridge, 1964). Annual rainfall averages 6000mm and mean annual temperature is 26°C (Holdridge, 1964; Vaughan et al., 2007; Wieme, 2011; Cornwell, 2012). Canals throughout the plantation provide irrigation and drainage. Additional plant species intermixed with the cacao, including *Eucalyptus deglupta*, *Cocos nucifera*, *Leucaena leucocephala* and *Musa acuminata*, ensure that cacao trees rarely receive more than 3hrs of direct sunlight per day. The cacao (*Theobroma cacao*) agroforest within the plantation is at times home to parrots, macaws, two-and-three-toed sloths, ants, termites and wasps.

**Study system:** *Theobroma cacao* at FINMAC is planted with 3m spacing between all trees. Six distinct clones, referred to here as clones A, B, C, D, E and F, are cultivated at the plantation. The land was converted from livestock pasture to cacao plantation circa 1982, and has been organically managed since 1997 (Cornwell, 2012). The FINMAC farm is situated on mostly flat to slightly rolling ground on moist soil with moderate-to-high amounts of leaf litter. Cornwell (2012) worked at FINMAC to characterize and quantify soil quality and composition. She explained that soil moisture is maintained by subterranean drainage tubes, soil is fertilized with composted coffee husks, and organic herbicides have been applied intermittently. During the Cornwell (2012) study, the soil on the organic cacao plantation had...
a mean pH of 5.8. Mean cation exchange capacity in the agroforest soil is 22.6cmol+/L (Cornwell, 2012). For more detailed information regarding soil composition and quality, see Cornwell (2012).

**Test subject selection:** Twenty trees each of clones B, C, D and E were selected within a 100 x 260m block at FINMAC. Clones A and F were not included since their fruits had recently been harvested at the time of study. Trees were chosen by walking inwards from the beginning of each clone-row (from the main farm road), marking every other tree, and skipping those which did not have at least four healthy fruits longer than 15cm. For clones B and C, 20 trees meeting the guidelines were available within single clone-rows. For clones D and E, 10 trees were selected from each of two clone-rows because fewer trees meeting the guidelines were available within each row. Marked trees were encircled with bright orange twine wrapped around adjacent trees to the selected tree. Plantation workers were asked not to cut or touch fruits in marked trees.

**Treatments:** Five treatments were randomly assigned in equal frequency to trees from each clone using the random integer generator in the “R” base package (R Development Core Team, 2012) so that each treatment was applied to four trees per clone. In cases where clones were split between two rows, treatments were assigned at equal frequencies between the rows. Eighteen liters of each treatment were prepared 24hrs prior to application at room temperature in 18-l plastic screw-top tanks as follows: (1) nothing applied; (2) water with 700g soil (3) water (4) water with 10g Trich-Aid (a *Trichoderma* inoculum powder); and, (5) water, 10g Trich-Aid, and 700g soil. Treatments 2, 3, 4 and 5 contained 50ml of Limonoil, an agricultural treatment adhesion solution (www.grupocolono.com).

Soil was obtained from a nearby cow pasture, finely sifted, and sterilized in the plantation cacao-drying oven at 250°C for 25min. Water used was non-chlorinated, obtained from a spigot on the plantation. Trich-Aid is a powdered *Trichoderma* inoculum, manufactured by Bio-Tech (Guapiles, Limón, Costa Rica). Treatments were applied twice throughout the study period: 22 March and 9 April 2013, using a Carpi 18-l manually-operated backpack sprayer. Sprays were delivered at a rate of 1l•min⁻¹ for 60 ±20s per tree, wetting the entire trunk, branches, leaves and fruits from the ground up to 2.5m.

**Monitoring:** Fruits in each experimental tree were observed for monilia infection at twelve time points between 23 March and 26 April 2013: March 23 and 25, and April 4, 8, 10, 12, 15, 17, 19, 22, 24 and 26. At each time point the number of fruits greater than 15cm in length was first counted. The number of fruits longer than 15cm with monilia infection was then recorded using a four-stage infection scale (Fig. 1): (0) no infection, (1) infection entering, indicated by irregular discoloration and/or irregular bump, (2) infection consuming fruit, indicated by irregular brown to black patch, and (3) production of fine, thickly layered cream colored spores. Infected fruits were marked with brightly colored flagging tape. Fruits with stage 3 monilia infections were cut from their trees and thoroughly buried under a layer of leaf litter, and were notated as grade three infected fruits in all subsequent observations. Fruits with any degree of monilia infection at the first time point were excluded from analysis.

Mean incidence of monilia infection was calculated using the cumulative percentage of fruits which became infected throughout the course of the study. Mean degree of monilia infection was calculated by taking the arithmetic mean of degrees of monilia infection, zero to three, throughout time points, so as to provide a hypothetical mean degree of infection for any time point. Differences between mean incidence and mean degree of infection between treatments, clones and time points were estimated by parametric 2-way ANOVA using R software (R Development Core Team, 2012). *A posteriori* comparisons between means were
performed using Tukey “honestly significant difference” (HSD) tests with the “agricolae” package for R (De Mendiburu, 2013). Graphs were generated with the base R software package (R Development Core Team, 2012).

RESULTS

Impact of spray treatments: Treatment five, “Trichoderma plus soil” significantly reduced mean incidence of monilia infection by 11% and mean infection degree by 0.121 (p<0.05) (Table 1) as compared to the control (treatment one).

Differences between clones: When results are split by clone, treatment five continued to reduce mean incidence (Fig. 2A) and mean degree (Fig. 2B) of monilia infection in clones C, D and E, though the change is only statistically significant in the cases of clone C, for infection incidence, and clone E, for infection degree. In clone B, treatment four significantly reduced mean incidence and degree of monilia infection.

Infections over time: Examination of mean incidence of monilia infection over time, grouped by treatment (Fig. 3A) revealed that fruits under treatment five were significantly less infected than the control (treatment one) at every time point after 12 March 2013 and

Fig. 1. Guide to monilia infection grades. Top to bottom: characteristic examples of infections of grades one, two, and three.

TABLE 1
Monilia infection under each treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean incidence</th>
<th>Mean degree</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16.74a</td>
<td>0.206a</td>
</tr>
<tr>
<td>2</td>
<td>13.62ab</td>
<td>0.178ab</td>
</tr>
<tr>
<td>3</td>
<td>12.66ab</td>
<td>0.158ab</td>
</tr>
<tr>
<td>4</td>
<td>9.15ab</td>
<td>0.144bc</td>
</tr>
<tr>
<td>5</td>
<td>5.78b</td>
<td>0.085d</td>
</tr>
</tbody>
</table>

1. 1=hard control (nothing applied), 2=water, soil, and Limonoil, 3=water and Limonoil, 4=water, limonoil and Trichoderma, 5=water, soil, Limonoil and Trichoderma.
2. percent of fruits observed displaying any degree of monilia infection.
3. on the 0-3 scale of infection, as described in methods.
4. means with the same letter(s) are not significantly different (Tukey HSD test, p<0.05).
fruits under treatment four were significantly less infected than the control (treatment one) at every time point after 19 April 2013.

Differences between clones over time:
Grouping mean incidence of monilia infection by clone over time (Fig. 3B) revealed that fruits of clones C and D were generally significantly more likely to become infected by monilia than those of clones B and E.

DISCUSSION

Trichoderma sprays reduced monilia infection so greatly that its use to combat monilia pod rot in cacao within the study area
is supported. Krauss & Soberanis (2002) found a 5.2% reduction in mean incidence of monilia infection in eastern Peru with numerous periodic Trichoderma sp. sprays over a 4-mo period. Our reduction in monilia mean incidence of infection by twice (11%) that amount was surprising, since our study was only 35d in length with only two Trichoderma applications.

Regarding viability of soil as an additive, differences between treatments four and five provided a preliminary justification for the addition of soil to Trichoderma sprays as a natural aid to antifungal activity. In this study we propose that the soil added to some treatment sprays provided a nutrient source for Trichoderma, aiding in survival and thus improving its ability to fight monilia infection. However, there are also a number of alternate possibilities which remain to be tested. For instance, the soil may simply help the fungi stick to the

Fig. 3A. Mean incidence of monilia infection at each time point, grouping treatments. Verticle lines are ±1 Tukey 95% confidence intervals. FINMAC plantation, Pueblo Nuevo de Guácimo, Costa Rica. March-April 2013.

Fig. 3B. Mean incidence of monilia infection at each time point, grouping clones. Verticle lines are ±1 Tukey 95% confidence intervals. FINMAC plantation, Pueblo Nuevo de Guácimo, Costa Rica. March-April 2013.
tree and fruit surfaces. It is also possible that additional soil microbes, which were not killed in the heat-sterilization process, may have had an effect on monilia infection.

In addition to soil, it would be useful to test other nutrient sources, such as fly ash as an organic carbon source. Use of ash in sprays would certainly be cheap but may be difficult to prepare and deliver using a backpack sprayer. Many classic studies of *Trichoderma* sp. have grown cultures in dedicated fungal media such as potato dextrose broth (Hermosa et al., 2000) or malt extract broth (Castle et al., 1998). A high-volume solution of *Trichoderma* culture in pre-sterilized potato dextrose or malt extract broth could easily be inoculated, incubated to grow the fungus to a high concentration, and stored indefinitely. The concentrated stock could then be diluted and efficiently delivered as needed via manual or motorized backpack sprayers.

Given the variation in treatment response between clones, we propose that the genetic variation between different clones has the potential to affect their response to spray treatments. Clone B exhibited an unusually strong response to treatment four and a relative lack of response to treatment five in the reduction of monilia infection incidence and degree. The unique response of Clone B to treatment as compared to other clones suggests that genetic variation in cacao can affect its response to *Trichoderma*-based antifungal spray treatments.

Furthermore, clones B and E tended to experience significantly lower incidences of monilia infection without *Trichoderma* application, implying that genetic variation in cacao also can influence its resistance to monilia infection. However, genetically resistant clones may not have desirable cacao bean production or quality. Studies involving more clones should be performed in order to learn more about the variable response of cacao to monilia and *Trichoderma* throughout various clones. Furthermore, molecular and genetic work incorporating sequence comparisons between tested clones could be used to identify common genes associated with monilia resistance and *Trichoderma* response, thus aiding in the process of clone breeding and selection. Differences between clones in treatment response should be used to inform selection of clones in order to maximize crop yield.

Regarding management implications, though the addition of sterilized soil to a *Trichoderma* spray per treatment five did appear to improve the antifungal activity of this treatment, it was not efficient enough for use as a larger scale treatment, simply because the soil tends to clog the backpack sprayer. Furthermore, heating and sifting enough soil to spray an entire plantation would take a lot of time. One solution to the backpack sprayer problem might be to use motorized backpack sprayers with better filters. Yet the more viable solution would be to develop an organic nutrient additive which is more easily prepared and delivered, such as the broths proposed above.

The 11% reduction in monilia incidence, in only 35d, by treatment five as observed through the course of the study period is in itself economically intriguing. Given such an improvement, the plantation mean productivity of 1 500kg dried beans/ha (Cornwell, 2012) would rise by nearly 200kg/ha to 1 698kg/ha. Treating the entire 110ha plantation with *Trichoderma* and soil would thus increase total mean productivity of dried beans by 21 780kg.

It is important to note that current monilia losses of cacao pods of 25-30% or more per year are common on FINMAC farm. However, even more interesting is to remember this study was only 35d in length, where infection incidence decreased at a positive linear rate (Fig. 3A, 3B). Would infection incidence decrease even more over a 4-mo growing period with periodical *Trichoderma* applications? Could the normal 30% loss be eliminated over this time period? If so, the economic, ecological and social benefits of *Trichoderma* control of monilia, promoting expanded cacao production, would be nothing short of phenomenal.
ACKNOWLEDGMENTS

Some of the practices carried out in this study have been tested by Geovanny Herrera at FINMAC, including using a spray consisting of a mixture of Trichoderma with fly ash and soil as additional nutrients. Thanks also to Hugo and Hubertien Hermelink and all of FINMAC for providing us with necessary cacao trees and material resources. Many thanks to Skye Greenler for her help with field work and data collection. Thanks to the ACM for their financial, linguistic, and academic support. Finally, thanks to all of the people of Pueblo Nuevo de Guácimo for welcoming us into their community.

RESUMEN

Uso de aspersión fungicida de Trichoderma para reducir infección de frutos de Theobroma cacao por Moniliophthora roreri en el noreste de Costa Rica. El cacao (Theobroma cacao) es un cultivo comercial importante en los climas tropicales como los de América Latina. A lo largo de las últimas décadas la infección del cacao cultivado con Moniliophthora roreri, conocida comúnmente como “monilia”, ha dificultado la producción del cacao en América Latina de manera significativa. Algunos estudios han propuesto el uso del hongo Trichoderma sp. en tratamientos de control biológico para prevenir y reducir la infección por monilia. No obstante, pruebas realizadas con tratamientos por aspersión que contenían Trichoderma en cultivos de cacao agroforestales produjeron resultados diversos. Investigadores y trabajadores agrícolas han sugerido que la adición de tierra, cenizas volantes u otras fuentes de carbón a la aspersión de Trichoderma podría mejorar su eficacia en la lucha contra la monilia. Para probar la validez de estas sugerencias, diseñamos una serie de mezclas para la aspersión que incluyan cultivos de Trichoderma, tierra y todos los testigos necesarios. Aplicamos aspersiones a 80 árboles de cacao (20 árboles para cada uno de cuatro clones seleccionados anteriormente por su resistencia a la monilia) en la finca de cacao orgánico FINMAC en Pueblo Nuevo de Guácimo, provincia de Limón, noreste de Costa Rica durante marzo y abril de 2013. Se aplicaron cinco tratamientos (testigo, agua, agua con tierra esterilizada, agua con Trichoderma, y agua con tierra esterilizada y Trichoderma). Se aplicó cada tratamiento a cuatro árboles de cada clon. Medimos la tasa de incidencia de infección por monilia bajo cada tratamiento por aspersión durante 35d. La aplicación de dos aspersiones a los árboles completos con una mezcla de Trichoderma y tierra esterilizada redujo la tasa de incidencia de infección por monilia en 11% (p<0.05) en solo 35d, en comparación con el tratamiento testigo. Esta reducción en la pérdida de frutos de cacao representa un aumento de 198kg/ha de semillas secas sobre la producción media de 1500kg/ha, o un aumento total de 21780kg en toda la plantación de 110ha. Proponemos que el uso de tal aspersión fungicida a lo largo de todo el ciclo de cultivo (120d) produciría una disminución aún mayor de la tasa de incidencia de infección. La aplicación de este método de control fungicida tiene el potencial de revitalizar la producción de cacao en la región.

Palabras clave: Theobroma cacao, cacao, control biológico, Costa Rica, fungicida orgánico, monilia, Trichoderma.

REFERENCES


Ecotourism and primate habituation: Behavioral variation in two groups of white-faced capuchins (*Cebus capucinus*) from Costa Rica

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Abstract: The increase of ecotourism operations within Costa Rica during the last 20yrs has brought more and more humans into close, direct contact with several wildlife species. One of these species is the white-faced capuchin (*Cebus capucinus*), highly gregarious, and with exposure over time, willing to come into close vicinity of humans and their developments. Such contact has its advantages and disadvantages for the ecotourism industry. We observed white-faced monkeys in order to assess the impact of human presence and development on monkey behavior, with a focus on aggressive, affiliative, and foraging behaviors in Curú Wildlife Refuge (CWR), located in Puntarenas, Costa Rica, and to ascertain the degree of over-habituation of capuchin populations at CWR. Though there exists no discrete behavioral parameters that measure over-habituation, it can be defined as an extreme state of habituation in which non-human primates not only lose fear of humans, but also actively include humans in social interactions or treat them as a food resource. We used instantaneous focal animal and group scan sampling during 8wks in March and April 2012. Two groups (approximately 20-30 individuals each) of capuchins were observed; the first near the tourist development at the Southwestern area of CWR, representing a habituated population that regularly foraged, rested, and groomed in the presence of humans. The second, was observed in the Northeastern area of CWR, did not visit the center of human activity and exhibited fear of humans. The habituated group exhibited significantly fewer instances of threatened behavior in response to human presence (p<0.0001) than the non-habituated group, and spent significantly more time eating and foraging (p<0.0001). While the habituated monkeys at CWR may not be over-habituated, they could become that way as development, especially ecotourism, increases. Over-habituation is a problem that affects capuchins in certain ecotourism sites in Costa Rica. It is critical that the consequences of habituation be studied more carefully, primarily in areas where ecotourism operations draw visitors to wildlife habitats. Rev. Biol. Trop. 62 (3): 909-918. Epub 2014 September 01.

Key words: *Cebus capucinus*, Costa Rica, ecotourism, habituation, over-habituation, white-faced capuchin.

Historically, primatologists have largely ignored anthropogenic influences on nonhuman primate communities. Leading primatologists in the field have admitted to previously viewing humans as “pesky” and “insignificant” aspects of the environment. In recent years however, primatologists and biological anthropologists have shifted their thinking: humans are a critical factor in any primate environment and must be assessed for potential influence on primate health, behavior, and long-term viability, especially in cases where the primates are already assessed as threatened, endangered, or critically endangered.

To determine potential anthropogenic threats to primates, this paper focuses on habituation—the gradually lessened response to an outside stimulus—and over-habituation, defined here as the behavioral state in which a primate has not only lost fear of humans but also includes humans in social interactions or interacts with humans as food sources. The primary examination of this topic is framed within ecotourism, which theoretically attempts to
attract visitors to and educate them about pristine natural environments while simultaneously creating low-impact development, providing economic benefits to the community that lives with and protects wildlife, as well as promoting peaceful coexistence. Ecotourism in practice is often flawed because it is largely unregulated, but conceptually has the potential for success in wildlife conservation (Buchsbaum, 2004).

This paper represents a case study examining two groups of wild white-faced capuchins (*Cebus capucinus*) that inhabit Curú Wildlife Refuge and Hacienda, a small, private ecotourist site in Costa Rica. Behavioral differences between groups of capuchins are expected due to differential contact with humans and human development. In particular, behavioral differences regarding fear-related activity (e.g. monkey fear of humans) and foraging behavior (e.g. if monkeys spend more time occupied by fear-related behavior, they will spend less time foraging for food) are expected.

Despite the worldwide reputation of Costa Rica as a leader in ecotourism (Honey, 2003; Buchsbaum, 2004; Gall & Hobby, 2007) recent studies have revealed that ecotourism has been far less effective in protecting significant ecological resources or benefitting local communities than originally thought. Stem, Lassoie, Lee, Deshler, and Schelhas (2003) focused on Corcovado National Park in Costa Rica and reported “mixed” findings regarding the effectiveness of ecotourism as a conservation and community development tool. The study resulted in several recommendations regarding ecotourism, including that “ecotourism would be most effective as a component of a broader conservation strategy,” referring to the need for firmer and more transparent planning and policies on the national level (Stem et al., 2003). It should be noted that in comparison to other ways in which Costa Ricans financially sustain themselves (e.g. forest plantations/logging, cattle ranching, agriculture, etc.) ecotourism remains the most environmentally sustainable option. Despite the abundant issues with ecotourism, tourists continue to visit Costa Rica for opportunities to see and interact with its abundant wildlife. This paper examines the potential impacts of the interactions between humans and primates on the behavior patterns of white-faced capuchins in an ecotourism setting.

**MATERIALS AND METHODS**

**Study site:** Privately owned Curú National Wildlife Refuge and Hacienda (CWR) located in Puntarenas, Costa Rica (9°47′36.24″N, 84°55′21.36″W), is considered a successful endeavor in ecotourism, conservation, and wildlife and land management (Schutt & Vaughan, 1995). Curú Wildlife Refuge covers approximately 14.94km² and receives about 1,600mm of precipitation annually. Ninety percent of precipitation falls during the wet season, from May to November (IMN, 2012). In 1995, approximately 30% of CWR was altered environment, including pastures, forest plantations, living fence rows, and fruit plantations (Schutt & Vaughan, 1995). The unaltered 70% was made up of natural forests, including upland dry, lowland evergreen, mangrove and beach-marine forest. Curu Wildlife Refuge attracts both domestic and foreign tourists and researchers to its immense biodiversity and relatively accessible location. For the current study, the previously agrarian region of CWR is referred to as the ‘Finca Side’ and the forested portion of CWR as the ‘Ceiba Side’ due to the presence of ceiba (*Ceiba pentandra*) trees. Additionally, in the agricultural areas, natural riparian forests, palm-lined canals, and living fencerows formed vegetation corridors (Schutt, & Vaughan, 1995). For more detailed information about CWR, see Schutt and Vaughan, 1995 and www.curuwildliferefuge.com.

**Study species:** Our study species, known commonly as the white-faced capuchin, white-faced monkey, or white-headed monkey, belongs to the taxonomic subfamily Cebinae. Cebines, which also include squirrel monkeys (genus *Saimiri*), are easily recognizable due to their long-time use in the film industry, presence in the pet trade, and use in street performances.
as sources of income (Jack, 2010). Because of their large range and relatively dense populations, white-faced capuchins are a well-studied species (e.g., Hall, 2000; Rose, 2000; Williams, & Vaughan, 2001; Perry et al., 2003; Vogel, 2005; Crofoot et al., 2009). White-faced capuchins are a “least concern” species as determined by the IUCN, meaning they have been evaluated for threats to long-term survival of the species and appear not to be at risk (IUCN, 2012). They have a broad range from Honduras to Ecuador and can survive in a wide variety of habitats—from primary forests in the Neotropics, to highly fragmented secondary or successional forests, to semi-developed areas of human habitation (Hall, 2000; Van Hulle & Vaughan, 2009). Male capuchins range in size from 1.3-4.8kg ($\bar{x}=3.0$kg) and females range in size from 1.4-3.4kg ($\bar{x}=2.3$kg). Males typically weigh 19.5%-27% more than females. Social structure is multi-male multi-female with one alpha male and alpha female. Social relationships within troops, which average 16.4 members, are maintained primarily via affiliative behaviors such as grooming, continued proximity, and coalition formation between preferred partners (Jack, 2010).

Data collection: Two groups of white-faced monkeys were observed for this study, each consisting of 20-30 individuals. The Finca group foraged and spent diurnal hours in forest patches and corridors in the formerly agricultural part of CWR. Finca monkeys were located farther away from the center of human development and activity at CWR, and rarely entered into close proximity to humans other than myself. The Ceiba group visited the center of human development two to three times per day to eat fruit provided to them by the one of the owners of CWR. The center of human development was the primary area frequented by visitors who wanted to see monkeys. I recorded distance of each monkey group from the farm headquarters (main buildings) at 5min intervals during observation of each group (Rose, 2000).

Observations were daily alternated between the Finca or Ceiba groups over a period of 8wks in the field (Table 1). Whenever possible observations took place between 0600 and 1600hrs, with breaks for breakfast and lunch. Instantaneous sampling (focal and group scans) was employed based on the conclusions of Rose (2000).

Instantaneous focal animal and group scan sampling: For focal animal sampling, the adult monkey in clearest view was chosen and observed for 30min, recording his/her specific behavior at 60s intervals. A prepared ethogram developed by Rose (2000) was utilized which identifies specific behaviors, as well as groups of more general “Activity Classes” (Table 2). Alterations to the ethogram prepared by Rose (2000) were also made, placing “Alarm Call” under “Activity Class 4: Aggression toward Other Species.” “Activity Class 5: Eating” was also combined with “Activity Class 6: Foraging” for analysis because they are closely linked in overall activity budgets, and eating

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Days of Observation$^a$</th>
<th>Total Hours of Focal Data</th>
<th>Total Hours of Scan Data</th>
<th>Total Hours Collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finca</td>
<td>22</td>
<td>26.7</td>
<td>20.27</td>
<td>46.97</td>
</tr>
<tr>
<td>Ceiba</td>
<td>26</td>
<td>41.2</td>
<td>31.5</td>
<td>72.7</td>
</tr>
<tr>
<td>Total</td>
<td>42$^a$</td>
<td>67.9</td>
<td>51.77</td>
<td>119.67</td>
</tr>
</tbody>
</table>

$^a$ For the first 8d of observation, behavior was observed in both sides of CWR because the alternate-day system had not been initiated.

TABLE 1
Sampling intensity for each monkey group by number of days, and sampling method.
Curú Wildlife Refuge and Hacienda. March-April 2012


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and foraging behaviors may be easily confused during observation.

In the event of losing sight of the initial focal animal, the next adult monkey closest to the researcher was identified and observation continued with that individual for the duration of the interval. If the focal animal was moving but did not appear to be fleeing, it would be followed. If the focal animal attempted to flee, observations would be discontinued so as not to appear as a predator and potentially increase stress on the animal. If the entire troop traveled away, observation was stopped, the time of troop departure was recorded and behavioral observations would cease.

For group scan collection, rather than recording specific behaviors, the number of individuals exhibiting a behavior from a certain Activity Class were tallied, also at 60s intervals. For example, Activity Class 1 is “Affiliative Behavior,” meaning that if three monkeys were observed grooming one another, and two monkeys playing, “5” would be recorded under “Class 1,” rather than distinguishing specific behaviors.

**Equipment:** A GPS device (GARMIN, Etrex Venture, www.garmin.com) was used to record coordinates of the 13 data collection sites (five Ceiba and eight Finca sites). The five Ceiba sites were located on tourist trails and the center of human development. The eight Finca sites were located on maintenance trails that were typically unvisited by humans other than the occasional visit by a refuge employee. Binoculars were used to view monkeys high up in the trees and a wristwatch was used to time observation intervals.

Statistical analyses were performed to examine the relationship of monkey behavior towards human behavior or proximity to human activity. We estimated the difference in mean distances of each monkey group to farm/refuge headquarters with a parametric one-way ANOVA. We tested the independence of behavioral activity classes with monkey groups with a contingency table (Sokal & Rohlf, 1995). Additionally, I estimated differences in mean frequency of occurrence of behavioral activity “Class 4” between monkey groups with a parametric, one-way ANOVA. Variables were transformed with $\log_{10}$ if not homogeneous between groups (Sokal & Rohlf, 1995). Statistical tests were run with Statgraphics Centurion XVI (Statpoint Technologies, Inc., 2011).

**RESULTS**

A daily mean of 2.84hrs of observations were collected. Monkeys in the Ceiba group were observed for more hours because they were easier to find (Table 1).

**Monkey group distribution:** Mean distance from the farm/refuge headquarters was greater for the Finca group monkeys.
(\bar{x}=0.99\text{km}; \log_{10}\bar{x}=-0.025) than for the Ceiba group (\bar{x}=0.71\text{km}; \log_{10}\bar{x}=-0.056) (F=10.9; df=1, 130; p=0.0013) (Fig. 1).

**Focal Animal:** Frequencies of occurrence of the nine activity classes (Class 3 [Sexual Behavior] was omitted for lack of data) were dependent on monkey group (X²=1665, df=8, p<0.0001) (Fig. 2). Classes 1, 4, 5 & 6, and 7 were most relevant to my study objectives.

**Class 1: Affiliative behavior between individuals:** Mean number of affiliative behaviors (e.g. grooming, playing etc.) per 30-min interval was larger for the Ceiba group (\bar{x}=6.3) than for the Finca group (\bar{x}=4.5), but not significantly so (F=0.44; df=1, 50; p=0.51).

**Class 4: Aggressive behavior toward humans:** Mean log-transformed number of aggressive behaviors toward other species (e.g. physical displays, alarm calls etc.) per 30min interval was significantly greater for the Finca group (log₁₀\bar{x}=0.83) than for the Ceiba group (log₁₀\bar{x}=0.07) (F=39.04; df=1, 58; p<0.0001) (Fig. 3). Additionally, in the Finca group 478 instances of aggression were recorded, while only 15 instances were recorded in the Ceiba group.

**Classes 5 and 6: Eating and Foraging:** Mean frequency of occurrence of eating/foraging behaviors (e.g. eat, drink, search in substrate etc.) per 30min interval was greater...
for the Ceiba group ($\bar{x}=11.1$) than for the Finca group ($\bar{x}=4.27$) ($F=40.4$; df=1, 149; $p<0.0001$).

**Activity Class 7: Movement**: Mean number of movement-related behaviors per 30-min interval was greater for the Finca group ($\bar{x}=6.7$) than the Ceiba group ($\bar{x}=5.9$), but not significantly so ($F=1.08$; df=1, 149; $p=0.30$).

**Group scan**: The activity budgets of the Finca group and Ceiba group differed significantly, according to scan data ($X^2=5798$, df=8, $p<0.0001$).

**DISCUSSION**

The Ceiba monkey group was clearly more habituated to human presence than the Finca group. The relative lack of aggression and fear response toward other species, specifically humans, in the Ceiba group suggests that Ceiba monkeys do not fear humans as potential predators nor view them as dangerous competitors for resources. The activity budget I (SEW) gathered from the Ceiba group more closely matched behavioral data of Rose (2000) than did the activity budget of the Finca group. The slight discrepancy between the Ceiba group behavior and that of the monkeys Rose (2000) studied is most likely a result of personal methodological errors such as misidentifying behaviors. I (SEW) imagine that with more hours, the Ceiba group data would more closely resemble the data collected by Rose (2000).

The Finca group, on the other hand, clearly maintained fear of humans, which is why the monkeys in the Finca acted aggressively toward me in essentially every interval I (SEW) completed. The Finca group is clearly not habituated as evidenced by their high rates of aggression toward other species, and their general attention to humans. The Finca group data greatly differed from data obtained by Rose (2000), on fully habituated monkeys, showing Finca monkeys not as accustomed to human presence. This is to be expected as the Ceiba monkeys spent most of their day on commonly followed tourist trails or in the center of human activity. Because the Finca monkeys rarely come into contact with humans, it logically follows that they would view any human as a threat. Indeed, initial reaction to habituation in primates includes increased rates of vocalization and increased aggression (Turner, 2005).

It is unclear what the consequences are of increased habituation of the Ceiba monkey group, as far as monkey health, visitor safety, and future conservation. Though little data is available concerning long-term effects of habituating New World primates, some studies (Altmann & Muruthi, 1988) of the Great Apes suggest that habituation can have long-term
impacts on behavior, particularly feeding and social structure (Turner, 2005). Because the owner of CWR routinely feeds the habituated monkeys, it is possible that they will become over-habituated in the future. It is also difficult to control the number of individuals that become habituated, and it is possible for habituation to spread from a few individuals to an entire population (Turner, 2005). Because white-faced capuchin troops often overlap in home range, the spread of habituation is a distinct possibility and thus a subject of concern.

First, overly habituated monkeys can become a threat to human safety and human health. They can serve as disease vectors (Crofoot et al., 2009) and they can be physically aggressive (Hall, 2000). While the Ceiba monkeys did not necessarily express evidence of over-habituation, two accounts of personal attack by white-faced capuchins were observed. In both of these instances, all humans involved were acting neutrally and not attempting to excite the monkeys in any way. Both accounts involved large male chasing researchers. However, no humans or monkeys were harmed in either case. While these cases are only anecdotal, and therefore not appropriate for statistical analysis, they are still relevant.

Secondly, over-habituated monkeys can jeopardize their own health and safety by increasing exposure to objects associated with humans and development, such as moving vehicles, dangerous machines, and power lines. Additionally, if habituated monkeys enter areas of human development, they can be exposed to human litter such as medical waste and broken glass, which I (SEW) witnessed at CWR. Third, they can alter their behavior, which might have negative effects in the long-term regarding reproduction and foraging patterns (Altmann, & Muruthi, 1988). Finally, they can become bothersome to the point of causing economic loss. They can cause damage, and steal food or valuable objects.

Current consequences of over-habituated white-faced capuchins can already be observed at numerous tourist attractions at sites all around Costa Rica. At Punta Leona Hotel and Resort, located just North of Jacó in Puntarenas province, dozens of white-faced capuchins can be seen from the primary eatery of the hotel (Van Hulle, & Vaughan, 2009). The monkeys at Punta Leona have become habituated to the point that they have absolutely no fear of humans, which has resulted in their stealing of food directly from restaurant tables and closer interactions between humans and monkeys (Van Hulle, & Vaughan, 2009). In Manuel Antonio National Park, white-faced capuchins are, similarly, totally unafraid of humans, which has resulted in physical aggression towards humans, as well as theft of objects humans leave unattended (Hall, 2000).

At CRW, the white-faced capuchins are not as aggressive and fearless as those at Punta Leona or Manuel Antonio, but it is possible that with continued exposure to humans and human activity, in addition to receiving provisions, they could reach that point.

Westin (2007) suggested with mantled howlers in Suriname, that “although [howlers] may have been altering their behavioral patterns in response to tourist presence, they appeared not to be suffering behaviorally… this general monkey ability to remain flexible and adaptable in the face of habitat change or disturbance (in this case through tourism) mirrors that found in other populations of howlers, and at other sites” (Naveda et al., 2008). This may indeed be the case for white-faced capuchins in Costa Rica, but with very little long-term, multi-generational data investigating the effects of habituation, we cannot know for certain. Research into the topic of human-primate interaction, particularly in a tourist context, should be proactive, rather than reactive. Thus, while fully habituating white-faced capuchins is not wholly negative (it does, after all, allow primatologists an up-close look at social hierarchy and social learning), it does pose potential problems, especially if habituated monkeys are not monitored closely. Furthermore, as so little research is published on the process of habituation, because it is typically viewed as a “means to an end,” it is becoming critically
important to investigate the effects of habituation (Turner, 2005).

Over-habituation poses a problem for conservation. Ideally, both monkeys and visitors on protected lands remain safe. At CWR, the monkeys and humans appear to be able to inhabit the same area safely, which may be a result of the owner providing them with a consistent food source. However, while in the present moment CWR seems to handle habituated monkeys safely and well, it is important to remain vigilant in achieving conservation goals that primarily benefit the capuchin populations.

Perhaps the best example of primate management in relation to exposure to humans comes from India, where large populations of rhesus macaques (Macaca mulatta) have become a pressing issue in many urban areas. The sprawl of human development has overlapped with macaque populations for an extended period of time, becoming not only pests, but also actual dangerous threats. Rhesus monkeys in certain urban areas of India have become hyper-aggressive, biting and injuring dozens of people each day (Ekwal, Yahya, & Malik, 2002). In order to lessen the negative effects of monkey-human interactions, 1,300 individual monkeys were translocated to a protected wildlife area far away from human development. A post-translocated study showed that the monkeys had adapted to the new environment and seemed to exhibit no negative responses (Ekwal et al., 2002).

In Kenya, where semi-provisioned baboons near tourist lodges have become over-habituated to the point of being viewed as pests, removal of animals has been less successful (Altmann, & Muruthi, 1988). When part of a population had been eradicated from an area of human development, another population typically relocated to gain access to the semi-provisioned habitat. Thus, the only sure way to limit the conflict between the humans and primates is to limit the offending human behavior, rather than eradicate the primates (Altmann, & Muruthi, 1988).

Though the white-faced capuchins in Costa Rica are not as dangerous and threatening as the rhesus monkeys in Northern India, translocation of certain populations is a viable option. With extensive protected land, adaptable white-faced capuchins could be relocated to more isolated areas. While this would not undo more subtle consequences of over-habituation, it could prevent more serious, long-term consequences. Additionally, stricter rules limiting accidental or deliberate human provisioning would help prevent the negative effects of over-habituation.

In future studies in CWR, it would be informative to focus on the Ceiba monkey group in order to more clearly assess potential over-habituation. Because CWR is an ecotourist site, it draws humans and primates into the same physical space. This overlap leads to habituation of the non-human primates, and in the long term could lead to over-habituation. Currently, CWR is seen as a successful site of conservation, but data suggests that in the future, primates could become over-habituated without stricter mitigation of human contact. The application of ethnoprimitology—a field that combines quantitative assessment of primate behavior with qualitative assessment of how humans perceive the animals and their conservation—is critical for CWR. Even though white-faced capuchins are recognized as “non-threatened” this is not necessarily accurate. Anthropogenic influences can lead to change in primates over time, even in species that are officially described as “least concern” by the IUCN.

ACKNOWLEDGMENTS

Many thanks to Scott Legge for advice, support, and editing. Thanks go to Chris Vaughan for providing useful feedback on my proposal for the project and to ACM for assistance in facilitating field research. We thank the Schutt family at Curu Wildlife Refuge and Hacienda for providing lodging, and this research site.
RESUMEN

Ecoturismo y habituación de primates: variación en comportamiento de dos grupos de monos carablanca (Cebus capucinus) en Costa Rica. El aumento de actividades ecoturísticas en Costa Rica durante los últimos 20 años ha ocasionado que más y más personas entren en contacto directo y cercano con varias especies de vida silvestre. Una de estas especies es el mono carablanca (Cebus capucinus), que es muy gregario y, al pasar el tiempo, tiende a acercarse a los humanos y sus instalaciones. Tal contacto tiene ventajas y desventajas para la industria del ecoturismo. Observamos monos carablanca con el fin de evaluar el impacto de la presencia humana y la infraestructura en el comportamiento de estos animales, enfocando los comportamientos de agresión, acicalamiento y forrajeo para determinar el grado de habituación excesiva de las poblaciones de monos carablanca en el Refugio de Vida Silvestre Curú, Puntarenas, Costa Rica. Aunque no existen parámetros específicos de comportamiento para medir la habituación excesiva, se puede definir esta condición como un estado en que los primates no humanos no solamente pierdan su temor a los humanos, sino que también incluyen a las personas activamente en sus interacciones sociales o las consideran como una fuente de alimentación. Los monos carablanca fueron observados a través del muestreo instantáneo focal y por escaneo grupal durante ocho semanas de marzo y abril de 2012. Dos grupos (aproximadamente 20-30 individuos cada uno) fueron observados: uno cerca del desarrollo turístico en la parte suroeste del refugio, que representó una población habituada que de manera regular comía y se acicalaba en presencia de humanos; el segundo grupo, que se observó en el noreste del refugio, no visitaba el centro de actividad humana y mostraba temor hacia los humanos. El grupo habituado mostró significativamente menos ocasiones de comportamiento amenazante ante la presencia humana (p<0.0001) y empleó más tiempo comiendo y buscando alimento (p<0.0001). Aunque el grupo de habituados en Curú probablemente no tenga habituación excesiva, esta condición podría surgir más adelante, especialmente si aumenta el desarrollo, y tenga habituación excesiva, esta condición podría surgir más adelante, especialmente si aumenta el desarrollo, y en particular el ecoturismo. La habituación excesiva es un problema que afecta a los monos carablanca en ciertos sitios ecoturísticos de Costa Rica. Es de suma importancia que las consecuencias de la habituación excesiva se estudien más a fondo, principalmente en las áreas donde las actividades ecoturísticas atraen visitantes a los hábitats de la vida silvestre.

Palabras clave: Cebus capucinus, Costa Rica, ecoturismo, habituación, habituación excesiva, mono carablanca.

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Species richness and relative species abundance of Nymphalidae (Lepidoptera) in three forests with different perturbations in the North-Central Caribbean of Costa Rica

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Abstract: Measurements of species richness and species abundance can have important implications for regulations and conservation. This study investigated species richness and abundance of butterflies in the family Nymphalidae at undisturbed, and disturbed habitats in Tirimbina Biological Reserve and Nogal Private Reserve, Sarapiquí, Costa Rica. Traps baited with rotten banana were placed in the canopy and the understory of three habitats: within mature forest, at a river/forest border, and at a banana plantation/forest border. In total, 71 species and 487 individuals were caught and identified during May and June 2011 and May 2013. Species richness and species abundance were found to increase significantly at perturbed habitats (p<0.0001, p<0.0001, respectively). The edge effect, in which species richness and abundance increase due to greater complementary resources from different habitats, could be one possible explanation for increased species richness and abundance. Rev. Biol. Trop. 62 (3): 919-928. Epub 2014 September 01.

Key words: butterflies, disturbance effects, edge effect, nymphalidae, species abundance, species diversity, species richness, Chiquita Nature and Community Project, Nogal Private Reserve.

Biological diversity has become increasingly important in the realm of conservation biology. Changes in species richness and species relative abundance due to disruptions in continuous habitats have been used in multiple studies to suggest or evaluate management decisions on which conservation efforts are needed to maintain the survival of a species or a community of species (Horner-Devine, Daily, Ehrlich, & Boggs, 2003; Uehara-Prado, Brown, & Freitas, 2007; Sjödin, Bengtsson, & Ekbom, 2008; Hjältén et al., 2012).

Insects, and in particular butterflies, have contributed greatly to understanding tropical biodiversity (DeVries, Alexander, Chacon, & Fordyce, 2012). Although plentiful, insects are still incredibly prone to great population increases and decreases, and have been used frequently as important indicators of disturbance to ecosystems (Erhardt, 1985; Fleishman, Thomson, Nally, Murphy, & Fay, 2005; Bobo, Waltert, Fermon, Njokagbor, & Mühlengber, 2006; Pöyry et al., 2006; Leidner, Haddad, & Lovejoy, 2010). Habitat loss is thought to be the greatest cause for insect extinction, and their populations can be easily influenced by anthropogenic forest fragmentation (Hogue, 1993; Leidner et al., 2010). Barlow, Overal, Araujo, Gardner, & Peres (2007) found that butterfly species richness in Brazil tended to be greater in primary forest than on Eucalyptus plantations, but that relative species abundance of the species existing there tended to be greater on the Eucalyptus plantations. Uehara-Prado et al. (2007) compared primary forest and fragmented forest in the Brazilian Atlantic, finding that butterfly species richness increased at fragmented sites while relative
abundance did not change, suggesting that forest fragmentation and disturbance does not
negatively affect species diversity. Horner-Devine et al. (2003) found that coffee farms
within 1–2.5 km of a forest reserve had greater butterfly species richness and relative species
abundance than both, farms 6–9 km away and within the forest reserve itself, indicating that
nearby forest may have a positive impact on butterfly species richness and relative species
abundance. It also suggests that forest edges may experience increases in species richness
and abundance.

This study focuses on the butterfly family Nymphalidae, which contains more species
than any other butterfly family in the world, except the family Lycaenidae. Nymphalidae
species can be found on every continent except Antarctica, although they are most diverse in
Neotropical regions (DeVries, 1987). More specifically, this study looked at fruit-feeding
nymphalids, a feeding guild in Nymphalidae, which feed on rotten fruit juices (DeVries et al., 2012). The objectives of this study were to
compare species richness and relative species abundance in the butterfly family Nymphalidae
in both the canopy and the understory between (a) mature forest habitat and two disturbance
habitats: (b) river/forest (a natural disturbance) and (c) banana plantation/forest (an anthropo-
genic disturbance).

MATERIALS AND METHODS

Study site: This study took place primarily in Tirimbina Biological Reserve (referred to as TBR in this study), located in the second
district of La Virgen, Sarapiquí, Heredia Province in the North central-Caribbean area
of Costa Rica. La Virgen has a population of 8715 people, and is a rural area (Municipalidad de Sarapiquí-Secretaria Técnica de Gobierno Digital, 2012b). The TBR (345 ha) is located in the Sarapiquí River Basin of approximately 1923 km² in size. It is bordered by other private properties that altogether create 600 ha of continuous forest cover (Tirimbina Biological Reserve, 2010a). The TBR (10°24′ N and 84°7′ W) is at an elevation of 180–220 m above sea level and is made up of 345 ha of lowland rainforest. Mean annual temperature is 25.3°C with a mean high of 30.2°C and a mean low of 20.2°C. Mean annual precipitation is 3777 mm. Landscape inclines generally fall between 10–25% with rolling hills, although closer to creeks and the river, slopes can reach 60%. Soils are of volcanic origin. The Sarapiquí River forms the Western border of the reserve. Tirimbina falls within two Holdridge life zones, Humid Tropical Pre-Montane Forest and transition to Basal and Humid Tropical Forest. Primary forest makes up 85% of TBR while the rest is secondary forest (Tirimbina Biological Reserve, 2010b).

The data of the banana plantation/forest border was part of a long term study executed by Chiquita Nature and Community Project at Nogal Private Reserve and forest fragments around it. This fragment is located close to Puerto Viejo, Sarapiquí at 40–60 m elevation. Mean annual precipitation falls between 3712–4000 mm. The mean annual temperature is 25.8°C, and the vegetation corresponds to Holdridge Life Zone of Humid Tropical Forest (Barquero Villalobos, 2009). Although close to Puerto Viejo, the surrounding area of this plantation is rural with fragmented forest. In general, banana and pineapple farms are especially common in Sarapiquí (Municipalidad de Sarapiquí-Secretaria Técnica de Gobierno Digital, 2012a).

Data collection: In TBR, data was taken from a river/forest border along the Río Sarapiquí and from within the forest itself. In TBR, data was taken 5 d/wk for 3 weeks and 4 d the 4th week between 2 to 31 May. Data used from the banana plantation/forest border were taken 3–7 May and 31 May–5 June, 2011 by staff of Chiquita Nature and Community Projects as part of their long-term butterfly survey.

Traps were made out of mosquito netting held in a cylindrical shape with an open bottom and closed top approximately 30 cm in diameter and 1 m in height as described in DeVries (1987). Between 50 and 100 mL of rotten
banana were placed in a small plastic cup on a platform hanging about 5-8cm from the open bottom of the trap. Banana was mashed and left to sit about 1d before baiting. Traps were rebaited with banana only as necessary throughout the week. Traps were checked once/d (trap-day). All butterflies captured during a trap-day were counted by number of individuals of each species and identified using DeVries (1987). Collected specimens were given to the Instituto Nacional de Biodiversidad (INBio), Santo Domingo de Heredia, Costa Rica. Data on precipitation levels each day were obtained from TBR. Sampling from the banana plantation study was conducted in a similar manner.

Twenty traps were set up at the river/forest border throughout the entire study. For the first week of the study, 30 traps within the forest were used from another ongoing study. For the next 3wks, 20 different traps were set up and used to collect forest data. In each habitat, traps were located at 10 or 15 paired points with one trap in the canopy and one in the understory. Traps in both perturbed habitats were set up inside the forest near the border, though not necessarily right on the tree line.

Differences in: a) mean number of species captured per trap-day and b) mean number of individuals per species per trap-day were estimated between canopy and understory both within and between habitats with two-way parametric ANOVA. A posteriori comparisons between means were tested with Scheffe intervals. Parametric test assumptions were carried out before executing ANOVAS and procedures followed Sokal and Rohlf (1995). Statistical analyses were carried out with Statgraphics XVI software (Statpoint Technologies, Inc., 2011).

Brillouin diversity indexes and Brillouin evenness indexes were calculated using Microsoft Excel 2007 Diversity Addin. Uneven sample sizes were accounted for by calculating the diversity index for each trap-day and checking for significance through an ANOVA test with Statgraphics XVI (Statpoint Technologies, Inc., 2011).

A species accumulation curve was created to estimate the proportion of butterflies identified to the actual number of species in the population. Relationship between daily precipitation data versus daily captures of number of species and number of individuals was estimated with simple regression carried out with Statgraphics XVI (Statpoint Technologies, Inc. 2011). A regression was not done for the banana plantation/forest habitat due to differences in data collection on precipitation A “square-root-Y” model (best fit) was used in the mature forest habitat and a “double-squared” model (best fit) was used in the river/forest border habitat for number of species. A “square root-Y” model (best fit) was used in the forest habitat and a “squared-Y” model (best fit) in the river/forest border habitat for number of individuals. Simper Analysis was used to evaluate changes in species composition with the PAST program (Hammer, Harper, & Ryan, 2001).

RESULTS

In total, 71 species and 487 individuals were caught and identified (Table 1). In general the three most abundant species were *Cithaerias pireta pireta*, *Hamadryas laodamia saurites*, and *Caligo brasiliensis sulanus*. When comparing the five most abundant species in each habitat, only two species were found in more than one habitat: *Catonephele orites* and *Colobura dirce dirce* (Table 2). At the genus level, only three were found in two habitats: *Hamadryas* (forest and river), *Catonephele* (forest and banana plantation), and *Colobura* (forest and banana plantation).

A total of 36 species were only found in one habitat: 8 in mature forest habitat, 21 in the river/forest border habitat and 7 in the banana plantation/forest border habitat (Table 3). For 20 of those species, only one individual was found.

Comparison of number of species: Mean number of species captured per trap-day was greatest in the banana plantation/forest border habitat ($\bar{x}$=0.74) and least in mature forest.
habitat ($\bar{x}=0.254$) with the river/forest border habitat as an intermediary ($\bar{x}=0.511$) ($F=29.1$; df=2, 997; $p<0.0001$). Overall, mean species number captured per trap-day was greater in the canopy ($\bar{x}=0.626$) compared to the understory ($\bar{x}=0.379$) when observations of all habitats were combined ($F=24.8$; df=1, 994; $p<0.0001$). However, this trend was not the same for each individual habitat ($F=16.07$; df=2, 994; $p<0.0001$, Fig. 1). The river/forest border habitat had the greatest difference between mean number of species between the canopy and the understory and no difference existed for the banana plantation/forest border habitat. Mean species number was greater in canopy than understory in mature forest habitat, but not significantly so. The lack of parallel trends in figure 1 indicates that habitat plays a strong impact on changing differences between mean number of species in the canopy and understory.

**Species accumulation curve:** The number of species accumulated in mature forest appeared to begin to plateau towards the end of the last sampling period, indicating that this study may have identified the majority of Nymphalidae species in that habitat. However,
TABLE 3
Number of individuals of each species found in only one habitat.

<table>
<thead>
<tr>
<th>Species found in only one habitat</th>
<th>Species Name</th>
<th>No of Ind*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forest</td>
<td>Callicore astarte patelina</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Consul panariste jansoni</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Dulcedo polita</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Hamadryas amphinome mexicana</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Morpho deidamia polybaptus</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Morpho menelaus amathonte</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Opsiphanes invirae cuspidatus</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Opsiphanes quiteria panamensis</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Total Number of Species</td>
<td>8</td>
</tr>
<tr>
<td>River/forest border</td>
<td>Caligo ilioneus oberon</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Callicore texa titania</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Chloreuptychia arnaca</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Cissia terrestris</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Consul fabius cecrops</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Ectima erycinoides erycinoides</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Fountainea halice chryosophana</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Fountainea ryphea ryphea</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Hamadryas feronia farinulenta</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Memphis arginussa eubaena</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Memphis elara</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Memphis glauce centralis</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Memphis lyceus</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Memphis morvis boisduvali</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Memphis oenomais</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Opsiphanes tamarindi tamarindi</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Pareuptychia metaleuca</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Pareuptychia ocurrhoe ocurrhoe</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Siderone galanthis galanthis</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Temenis laothoe agatha</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Total Number of Species</td>
<td>21</td>
</tr>
<tr>
<td>Banana plantation/forest border</td>
<td>Agrias amydon philatelica</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Cissia pseudoconfusa</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Eryphanis lycomedon</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Megeuptychia antonoe</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Memphis proserpina proserpina</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Myscelia leucocyana smalli</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Zaretis itys itys</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Total Number of Species</td>
<td>7</td>
</tr>
</tbody>
</table>

*Number of Individuals.
in both the river/forest habitat and the banana plantation/forest habitat, the number of identified species appeared to continue sloping upwards, indicating that there may still have been several unidentified species, leaving the final data incomplete.

Comparison of number of individuals:
Mean number of individuals was greatest in the banana plantation/forest border habitat ($\bar{x}=0.875$) and least in the mature forest habitat ($\bar{x}=0.263$) with the river/forest border habitat as an intermediary ($\bar{x}=0.534$) ($F=32.3$; df=2, 997; $p<0.0001$, Fig. 2). Overall, mean number of individuals captured per trap-day was greater in the canopy ($\bar{x}=0.650$) than the understory ($\bar{x}=0.467$) when observations of all habitats were combined ($F=9.93$; df=1, 994; $p=0.0016$), although the banana plantation/forest border habitat strayed from this pattern when looking at each habitat ($F=19.2$; df=2, 994; $p<0.0001$, Fig. 3). The river/forest border was the only habitat with a significant difference between the mean number of individuals in canopy and understory. The lack of parallel trends in figure 3 indicates that habitat plays a strong impact on changing differences between mean number of individuals in the canopy and the understory.

Brillouin and Brillouin Evenness Diversity indices: The Brillouin Diversity Index indicated significantly different mean indices between habitats. The banana plantation/forest habitat had the greatest diversity ($\overline{H}_B=0.082$), the mature forest habitat had the smallest diversity ($\overline{H}_B=0.013$), and the river/forest border habitat...
was intermediary ($H_B=0.055$). The Brillouin Evenness Index had similar results; the banana plantation/forest habitat had the greatest index ($H_B=0.18$), the river/forest border as intermediary ($H_B=0.12$), and the mature forest habitat with the smallest index ($H_B=0.35$).

Simper Analysis revealed that *Cithaerias pireta pireta* contributed the most to dissimilarity between groups (% Contribution=7.769%) with a relatively great abundance in the banana plantation/forest border habitat (mean abundance=0.215) and relatively smaller abundances in the river/forest border habitat (mean abundance=0) and in the mature forest habitat (mean abundance=0.00941). *Hamadryas laodamia saurites* (Contribution %=3.094%), *Caligo brasiliensis sulanus* (Contribution %=2.87%), and *Nessaea aglaura aglaura* (Contribution %=2.858%) also had greater contributions to dissimilarity.

**DISCUSSION**

Species richness and species abundance increased in the habitats of disturbance, with the greatest numbers in the banana plantation/forest border habitat. Brillouin Diversity Index and Brillouin Evenness Index indicated greater butterfly diversity in habitats of disturbance as well. Species richness and abundance tended to be greater in the canopy than in the understory. Previous studies have indicated that borders between two different kinds of habitat can attract or repel different butterfly species, creating something called “edge effects”. According to Ries, Sisk, and Karban (2008), butterfly density tends to increase at the border between two habitats when there is a strong difference between habitat quality and distribution of necessary or complementary resources. DeVries (1987) attributed this increased species richness and relative species abundance at forest edges to similarities between canopies and forest edges, such as more light and a more dynamic and diverse physical environment. Tree gaps within continuous rain forest may also experience canopy butterfly species near the ground due to changes in vegetative structure (Pardonnet, Beck, Milberg, & Bergman, 2013), reaffirming that areas of disturbance may contain similar characteristics to the canopy, and thus attract more of those species. Horner-Devine et
al. (2003) hypothesized that increases near borders could be due to increased microclimates that include necessary host plants and food, as well as increased space for movement.

Of the 71 species identified, 36 (50.7%) of those species were found in only one habitat. Of the five most abundant species found in each habitat, only two in total were found present in more than one habitat. The top contributors to dissimilarity according to the Simper Analysis were also all species within the five most abundant in each habitat. This suggests that each habitat provides key services to specific species and has strong effects on species composition, even though each of these habitats includes primary forest and all are found in the same region. Pardonnet et al. (2013) found that tree gaps in Peruvian rain forests caused changes in butterfly assemblages when compared to adjacent nearby understory, indicating that species composition may change within the same continuous forest should vegetative structure vary, as it does at habitat borders.

Increased precipitation negatively affected the number of species and individuals significantly in mature forest habitat. Although not significantly affected, number of species and individuals did tend to decrease with increased rainfall in the river/forest border habitat as well. This could be because rainfall is not conducive to flight (Raupp, 2006). Precipitation levels could account for high levels of variance in samples. It also suggests that species abundance is affected by more than just habitat.

Limitations to my study may include error generated from temporal and location differences in data collection. Data from the banana plantation/border habitat came from a study site 20km from TBR and was collected 2yrs before the data collected for the other two habitats. In the first week of data collection in the mature forest habitat, traps were set up in different locations than in weeks 2-4 due to a lack of available traps, creating more chance for error. This study was also conducted in May, during which the dry season usually transitions into the rainy season, which may have affected changes in butterfly species and abundance.

Future research may want to account for changes in precipitation levels and changes in species composition over increased time periods. May tends to be the transition between the rainy season and the dry season in Sarapiquí, and there might be differences between rainy season data and dry season that are worth comparing, especially because commonality of some species seemed to change from the beginning of the month to the end. Therefore, results may vary depending on the time of year. As indicated by the increasing curves in figure 4, a
longer sampling period may also be needed to obtain a complete picture of species composition in each habitat.

ACKNOWLEDGMENTS

Special thanks to Christian Miranda for teaching me all about butterfly identification and accompanying me so many times in the field. We thank the Chiquita Nature and Community Project for allowing us to use data from their own research project. We also thank Isidro Chacón for helping us with species identification and everyone at the ACM office, most especially Michael McCoy and Chris Vaughan for guidance on our project and review of this paper, as well as Mario Morera for helping the first author in Spanish translations.

REFERENCES


Beach almond (*Terminalia catappa*, Combretaceae) seed production and predation by scarlet macaws (*Ara macao*) and variegated squirrels (*Sciurus variegatoides*)

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Abstract: Knowledge of ecological impacts of exotic beach almond (*Terminalia catappa*) in the central Pacific of Costa Rica are little known, but studies have found this species to be a potentially important food source for endangered scarlet macaws (*Ara macao*). In this study, reproductive phenology and seed predation by variegated squirrels (*Sciurus variegatoides*) and scarlet macaws were measured during March and April 2011 on beaches of central Pacific coastal Costa Rica. Seed productivity and predation levels were quantified on a weekly basis for 111 beach almond trees to assess the importance of beach almond as a food source for scarlet macaws and the extent of resource partitioning between seed predators. Seed production of the trees was great (about 194 272 seeds) and approximately 67% of seeds were predated by seed predators. Macaws consumed an estimated 49% of seeds while squirrels consumed 18%. Additionally, evidence of resource partitioning between squirrels and macaws was found. Scarlet macaws preferred to feed on the northern side and edge of the canopy while squirrels preferred to feed on the southern and inside parts of the canopy. Both species ate most seeds on the ocean side of the tree. Despite the status of this tree as an exotic species, the beach almond appears to be an important resource for scarlet macaw population recovery. The resource produced by this tree should be taken into account as reforestation efforts continue in Costa Rica. Rev. Biol. Trop. 62 (3): 929-938. Epub 2014 September 01.

Key words: *Ara macao*, beach almond, Costa Rica, phenology, scarlet macaw, seed predation, *Sciurus variegatoides*, *Terminalia catappa*, variegated squirrel.

The role of, and response to, exotic species has recently become a hotly debated topic (Davis et al., 2011). While exotic species are often viewed as detrimental to native communities, it is important to consider what the actual effects of an exotic species are in this age of rapid environmental change. In many cases introduced species do indeed result in negative environmental changes (Vitousek, D’Antonio, Loope, & Westbrooks, 1996), but in some cases, there is evidence that an introduced species can improve conditions for some native species. Changes generated by introduced species such as pest control, pollination, competitive and predator release, and trophic subsidies can result in benefits to native species, especially in environments where climate and land use is changing rapidly, resulting in modified native communities (Rodriguez, 2006; Schlaepfer, Sax, & Olden, 2010).

In this case, the scarlet macaw (*Ara macao* L.) in Costa Rica, suffered severe population declines in the 1980s and early 90’s due primarily to deforestation and poaching (Wiedenfeld, 1994; Vaughan, Nemeth, Cary, & Temple, 2005). This population decline resulted in the listing of scarlet macaws as threatened on the Appendix I of the Convention on International Trade in Endangered Species (CITES). However, over the last 20 years, habitat restoration,
local education, and poaching control have resulted in considerable growth of the Costa Rican scarlet macaw population (Vaughan et al., 2005). As the macaw population has been recovering, much about their behavior and ecology has been investigated, including diet (Vaughan, Nemeth, & Marineros, 2006). Most notably, recent macaw diet studies have indicated a large reliance on exotic species, with 52% of their diets consisting of exotic species while one specific exotic tree, Terminalia catappa (L.) makes up about 36% of the diet (Matuzak, Bezy, & Brightsmith, 2008).

Tropical forest, especially dry forest in Central America has been has been severely reduced primarily by deforestation for cattle grazing (Quesada & Stoner, 2003). When grazing occurs, forests have less regeneration of many native trees that macaws feed on (Stern, Quesada, & Stoner, 2002) and as this reduction in native food sources has occurred, T. catappa has been widely planted in reforestation efforts, especially along beaches (Vaughan et al., 2006).

Terminalia catappa is native to Southeast Asia but, due to ocean currents and humans, it has spread to almost all tropical areas. While T. catappa is common throughout Costa Rica, high tolerance to wind and salt stress makes it most prevalent on beaches. On beaches, T. catappa is known to prevent erosion of beach sand, provide food and shelter for humans and animals, and has potential for commercial cultivation for its seeds (Thomson & Evans, 2006). Based on observations, the seed production peak of T. catappa coincides with that of many other dry forest species, occurring during the dry season (January-May) (Frankie, Baker, & Opler, 1974).

Scarlet macaws, as seed predators, are also important in the reproduction of T. catappa. In addition to macaws, variegated squirrels (Sciurus variegatoides Ogilby, 1839), ants, and various bats are known to feed on T. catappa seeds (Zuchowski, 2007). Of these other seed predators, squirrels are the most common (personal observation). In utilizing the same resource as scarlet macaws, it would be expected that these predators would interact in some way, either competing for or partitioning the resource (MacArthur, 1958).

The objective of this study was to investigate the role and the effects of an exotic species as a food source for threatened scarlet macaws in Costa Rica and how interaction with variegated squirrels affects macaw use of the resource. Specific objectives were to investigate 1) production and predation levels of T. catappa seeds during the dry season and 2) seed predator feeding patterns and interactions on T. catappa trees.

MATERIALS AND METHODS

Study site: This study was performed on the central Pacific coast of Costa Rica between the Tárcoles River and the resort Punta Leona (9°45’ N - 84°40’ W). The study area is characterized by the transition between dry and humid tropical forest (Tosi, 1969), but is now dominated by agriculture and small forest patches. The climate is warm and humid, with a mean annual temperature of 25-30°C and mean annual precipitation of 2.5-3.3m and distinct wet (May-December) and dry (January-April) seasons (Coen, 1983).

Seven beaches were studied; Playas Azul, Tárcoles, Pita, Agujas, Limoncito, Manta, and Blanca (Fig. 1). Playas Azul, Tárcoles, and Pita are primarily residential, while playa Agujas has a section developed for tourism while most is unoccupied by humans. Playas Limoncito, Manta, and Blanca are part of the resort Punta Leona and playas Manta and Blanca are frequented by resort guests, while Limoncito is small and isolated, with little human presence.

Tree selection and characteristics: Judgment sampling was used to get a wide array of trees of different sizes, locations, and surrounding characteristics. Number of trees selected per beach varied by beach length and was as follows; Azul (n=14), Tárcoles (n=20), Pita (n=20), Agujas (n=24), Limoncito (n=5), Manta (n=16), and Blanca (n=12). Both the total tree height and the height of the leaved canopy
(total height - height of trunk without leaves) were measured with a clinometer. Additionally, a tape measure was used to measure the crown diameter (distance from edge to edge of the crown). A combination of crown height and diameter was used to calculate crown volume.

**Seed production data:** Seed production was monitored on a weekly basis on all trees studied. To quantify the reproductive phenology, the number of mature seeds, immature seeds, and flower heads were counted in three randomly selected sections of approximately 1 m³ per tree. A mature seed was defined as a seed the size where predators were likely to consume it, determined by observations of seeds already eaten. Immature seeds were seeds smaller than the seeds frequently consumed by predators. Flower heads were defined as any flowering head containing flowers with petals.

**Seed predation data:** Seed predation was measured on a weekly basis in 0.5 m² plots. Eight plots per tree were organized with four plots on the interior, approximately 0.5 m from the center of the crown and four plots around the edge of the crown. Plots were oriented North, South, East, and West, with two plots in each direction (Fig. 1). Plots 1, 3, 5, and 7 were considered interior plots while plots 2, 4, 6, and 8 were considered exterior plots. Plots 1 and 2 were considered North plots, plots 3 and 4 were considered East plots, plots 5 and 6 were considered South plots, and plots 7 and 8 were considered West plots. Preliminary trials indicated that there was negligible post-dispersal predation of entire seeds or empty shells, and that the exocarp of the seeds remained green for approximately one week. Thus, only seeds with some green exocarp left on their outer shell were considered during weekly sampling.

Seeds from each plot were categorized by 5 seed predation classes based on shell markings. These classes included; macaw predation, macaw rejection, squirrel predation, squirrel rejection, and no predation. Predated seeds were defined as opened seeds where the embryo had been eaten (Fig. 2, Fig. 3, Fig. 4 and Fig. 5). Rejected seeds were defined as seeds showing evidence of being picked by a predator, but not opened to expose the inner part (Fig. 6, Fig. 7). No predation seeds were defined as those that showed no sign of predation. Macaws and squirrels each had two methods of opening seeds (Fig. 2, Fig. 3, Fig. 4 and Fig. 5).

Total seed production and predation were calculated for each tree based on the seed, flower, seed predation, and seed rejection density obtained during sampling, combined with the total volume of the crown and area under the crown for each tree. For analytical purposes, the no predation category consisted of seeds that were rejected by predators and seeds that showed no signs of predation.

Contingency tables and Chi-squared tests (Sokal & Rohlf, 1995) were used to analyze trends in preferred feeding location of seed predators along with the association between
Fig. 2. Scarlet macaw (*Ara macao*) predated fruits (type 1). The fruits have large, smooth chunks taken out of the top half in a messy and imprecise way.

Fig. 3. Scarlet macaw (*Ara macao*) predated fruits (type 2). The fruits are split in half and often most of the exocarp has been removed. Method of opening is messy and imprecise.

Fig. 4. Variegated squirrel (*Sciurus variegatoides*) predated fruits (type 1). The fruits are mostly if not completely free of exocarp. There are many small grooves which were made by the teeth of the squirrel. Opening to seed is often not directly on top.

Fig. 5. Variegated squirrel (*Sciurus variegatoides*) predated fruits (type 2). The fruits have been carefully opened on the top. Small grooves made by the squirrel’s teeth can be seen.
RESULTS

Seed production and predation: Across 111 trees, approximately 194,272 mature seeds were produced during the 2-mo study period ($\bar{x}$=1,854 seeds/tree). A total 24,294 seeds were predated by scarlet macaws, 9,857 seeds rejected by macaws, 8,814 seeds predated by squirrels, 2,448 seeds rejected by squirrels, and 5,419 seeds that fell without signs of predation during the study period. This makes for a total of 50,832 seeds that fell from trees during the study period, 31% of the total mature seeds produced. Of those that fell, 33% had not been consumed by predators. The proportion of seeds that fell varied by beach, with Northern beaches (Azul and Tárcoles) having a higher proportion of seeds produced that fell compared to other beaches. However, Azul differed from Tárcoles in that a higher proportion of fallen seeds were predated (Table 1).

Predator preference and interaction, beach: Both the frequency and amount of predation varied significantly by beach for macaws and squirrels. Macaw predation was more frequent than expected on Azul, Pita, Limoncito, and Manta and less frequent than expected on Tárcoles and Agujas ($X^2=48.7$, df=6, $p<0.001$) (Fig. 8A). Squirrels, on the other hand, preferred to feed on Azul and Tárcoles beaches while feeding less frequently than expected on the more Southern beaches ($X^2=15.8$, df=6, $p=0.01$) (Fig. 2A). Notably, macaws and squirrels differed in feeding preferences on Tárcoles (squirrel preferred), Pita, Limoncito, and Manta (macaw preferred) beaches and were similar on Azul, Agujas, and Blanca. Seeds that were not predated were found more often than expected on Tárcoles, Limoncito, and Manta and less frequently on Agujas ($X^2=21.9$, df=6, $p=0.001$) (Fig. 8A).

When predation did occur, the amount varied by beach for each seed predator. For macaws, significantly more seeds were consumed on Southern beaches (Agujas, Limoncito, Manta, and Blanca) while fewer seeds were consumed on the Northern beaches (Azul, Pita, Limoncito, and Manta) compared to Southern beaches. For squirrels, seeds were consumed more frequently on Southern beaches compared to Northern beaches.
Tárcoles, and Pita) (Table 2). Squirrel predation was consistently less than macaw predation and significantly more predation occurred on Manta compared to Azul and Pita beaches. Significantly more intact seeds fell on Agujas and Blanca compared to Azul and Pita beaches (Table 2).

Tárcoles was the only beach where macaws didn’t consume approximately 50% of the predated seeds. Squirrels, on the other hand, consumed a very low proportion of predated seeds on Agujas and Limoncito compared to other beaches (Table 1).

**Predator preference and interaction, plot:** There were fewer significant differences in predation frequency by plot. Observed macaw predation frequency was not significantly different than expected ($X^2=10.5$, df=7, $p=0.16$), although there was a tendency to feed more often above plots 2, 7, and 8, and feed less frequently above plots 3, 4, and 6. Squirrel predation frequency also did not vary significantly from expected ($X^2=10.5$, df=7, $p=0.16$).

---

**Table 1**

*Terminalia catappa* mature seed production and predation during study period

<table>
<thead>
<tr>
<th>Beach</th>
<th>Total Mature Seeds</th>
<th>Mature Seeds per Tree</th>
<th>Total Fallen Seeds</th>
<th>Fallen Seeds</th>
<th>Macaw Predation</th>
<th>Squirrel Predation</th>
<th>No Predation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azul</td>
<td>9976</td>
<td>713</td>
<td>6148</td>
<td>62</td>
<td>50</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Tárcoles</td>
<td>22649</td>
<td>1132</td>
<td>9888</td>
<td>44</td>
<td>39</td>
<td>18</td>
<td>43</td>
</tr>
<tr>
<td>Pita</td>
<td>45961</td>
<td>2298</td>
<td>12757</td>
<td>28</td>
<td>49</td>
<td>17</td>
<td>34</td>
</tr>
<tr>
<td>Agujas</td>
<td>46404</td>
<td>1933</td>
<td>8061</td>
<td>17</td>
<td>47</td>
<td>10</td>
<td>44</td>
</tr>
<tr>
<td>Limoncito</td>
<td>16622</td>
<td>3324</td>
<td>3613</td>
<td>22</td>
<td>62</td>
<td>4</td>
<td>34</td>
</tr>
<tr>
<td>Manta</td>
<td>38887</td>
<td>2430</td>
<td>6493</td>
<td>17</td>
<td>50</td>
<td>21</td>
<td>29</td>
</tr>
<tr>
<td>Blanca</td>
<td>13774</td>
<td>1148</td>
<td>3873</td>
<td>28</td>
<td>48</td>
<td>28</td>
<td>24</td>
</tr>
<tr>
<td>All Beaches</td>
<td>194272</td>
<td>1854</td>
<td>50832</td>
<td>31</td>
<td>49</td>
<td>18</td>
<td>33</td>
</tr>
</tbody>
</table>

---

**Fig. 8.** Chi-squared test Pearson’s residuals for observed presence/absence of fallen seeds predated by macaws (black bar) and squirrels (gray bar) or showing no sign of predation (white bar) against expected frequency by A) beach and B) plot. Positive values indicate predation occurred more frequently than expected.
p=0.16) but there was a tendency to feed more frequently in plots 1 and 6-8 and less frequently than expected in plots 2-5. The observed frequency of intact seeds did differ significantly from expected (X2=21.9, df=6, p=0.001), with the most notable difference being a much lower frequency observed in plot 3 (Fig. 8B).

When macaws did feed, the amount of seeds eaten above each plot differed significantly, with higher amount of predation in plots 2 and 8 compared to plot 4. While not significantly different, there was also a tendency to eat more seeds in plot 6. Overall, macaws tended to consume more seeds around the edge of the canopy on the ocean side. Squirrel predation did not differ significantly by plot although there were slightly more seeds consumed above plots 1, 5, and 8. Plot was marginally significant in number of seeds that fell without predation (Table 2).

### DISCUSSION

Regarding scarlet macaw predation on beach almond, the high proportion of *T. catappa* seeds in scarlet macaw diets (Matuzak et al., 2008) resulted in the consumption of almost half of the seeds produced by *T. catappa*. This is very similar to a study by Villaseñor-Sanchez, Dirzo, & Renton (2010) that found a 43% seed predation rate by *Amazona finschi* on *Astronium graveolens* and a study by Coates-Estrada, Estrada, & Meritt (1993) that found a mean predation rate of 51% by *Amazona autumnalis* on *Stemmadenia donnell-smithii*. These examples are, however, much greater than other studies of seed predation by parrots. A study of scarlet macaw predation of Brazil nut (*Bertholletia excelsa*) revealed that macaws consumed only 10% of the seed crop (Trivedi, Cornejo, & Watkinson, 2004). The predation rate reported in this study, however, only includes seeds that had fallen and there were many seeds left to fall in most trees at the end of the study.

Macaws preferred to feed on three beaches; Blanca, Manta, and Limoncito. All three of these beaches are located within a resort/club called Punta Leona, but are the furthest beaches from the macaw roosting site in the Guacalillo Mangrove Reserve (Vaughan et al., 2005) to the north of the study area. This would not be expect based on optimal foraging theory (MacArthur & Pianka, 1966), but could be a result of the conservation efforts made by Punta Leona. The resort has planted many *T. catappa* trees on its grounds and throughout the whole central Pacific coast of Costa Rica. Additionally, the beaches of Punta Leona are part of the ecological blue flag program in Costa Rica, which is only awarded to the healthiest beaches of Costa Rica.

#### TABLE 2

<table>
<thead>
<tr>
<th>Plot</th>
<th>Macaw</th>
<th>Squirrel</th>
<th>No Predation</th>
<th>Beach</th>
<th>Macaw</th>
<th>Squirrel</th>
<th>No Predation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.65&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.93</td>
<td>1.16</td>
<td>Azul</td>
<td>1.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.06&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>1.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.79</td>
<td>1.58</td>
<td>Tárcoles</td>
<td>1.32&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.98&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.11&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>1.62&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.66</td>
<td>0.99</td>
<td>Pita</td>
<td>1.77&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.99&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>1.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.83</td>
<td>1.11</td>
<td>Agüas</td>
<td>2.64&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.52&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>1.80&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.04</td>
<td>1.62</td>
<td>Limoncito</td>
<td>2.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.95&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.59&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>1.94&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.91</td>
<td>1.08</td>
<td>Manta</td>
<td>2.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.22&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>1.73&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.92</td>
<td>1.26</td>
<td>Blanca</td>
<td>1.68&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.85&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.70&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>2.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.19</td>
<td>1.40</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>F</td>
<td>3.74</td>
<td>0.95</td>
<td>1.97</td>
<td>–</td>
<td>7.15</td>
<td>3.15</td>
<td>4.38</td>
</tr>
</tbody>
</table>

Different letters (<sup>a,b,c</sup>) indicate significant differences between areas using Tukey tests (p<0.05).
Squirrels, on the other hand, had preferences for the most human-dominated beaches. Azul, Tárcoles, and Pita beaches are all residential, with houses adjacent to the beach. Squirrels are often associated with humans, so they are better adapted to the disturbances common on developed beaches. Interestingly, the number of macaw feeding events was not necessarily lower on residential beaches, but the number of seeds eaten was. This indicates that macaws spend less time feeding on residential beaches, even though they visit them about the same amount as other beaches. This was probably observed because macaws were more likely to be disturbed by human presence.

Regarding preferred feeding locations, both seed predators fed more often over the ocean side (plot 8) and less often away from the ocean (plot 4). This is likely because the main method of natural propagation of *T. catappa* is through the ocean currents and tides (Thomson & Evans, 2006), making it advantageous to produce a large number of seeds on the ocean side. This larger concentration of seeds would draw more seed predators. Plot 4, on the other hand, faces away from the ocean and is often engulfed by other trees, decreasing the light that reaches that side.

The preferred feeding locations in trees provide some evidence of resource partitioning between seed predators in this study. There was a weak division in predation between the north and south sides of the tree canopy, where macaws tended to feed on the northern side while squirrels preferred to feed on the southern side of the tree. This indicates that macaws may prefer to feed more often in the morning as they fly from their roosting site to the north of the study area. There was also a slight tendency for squirrels to prefer feeding on the inside of the tree canopy, while macaws tended to feed on the edge of the canopy. This resource partitioning is likely a result of morphological differences between species. Macaws fly from tree to tree landing on outer branches. Alternatively, squirrels tend to climb the trunk to access the crown, making it easier to consume seeds along the interior of the tree.

This study suggests that *T. catappa* seeds are a very important food resource for scarlet macaws during the study period (March-April, 2011). As macaw populations have been increasing in the central Pacific of Costa Rica (Vaughan et al., 2005), *T. catappa* has become an important part of their diet (Matuzak et al., 2008). Additionally, this study reveals high *T. catappa* seed predation by macaws along beaches. As a tree that grows quickly and produces many seeds, *T. catappa* planting projects, which are occurring throughout the Central Pacific of Costa Rica, appear to be important in macaw recovery. Additionally, *T. catappa* seeds are important for other seed predators, and current predation rates suggest that enough food is produced to support both squirrels and macaws.

This system is an example of how exotic species can interact with and influence native populations. In this case, this exotic species is likely integral in supporting an endangered species, and facilitating their recovery, while possibly beneficial to beach communities (Thomson & Evans, 2006). This does not, however, mean that the overall effects of *T. catappa* on the surrounding community should not be investigated to assess whether the species is harming other elements of the community.

The importance of Punta Leona resort as an example to other beaches, is shown by its success and, thus should be a model for other beaches. Despite being very populated, Punta Leona had the greatest macaw presence, which indicates that beach health and cleanliness is likely more important than human presence. Not only should reforestation of native trees along with *T. catappa* take place, but measures should be taken to clean up beaches and reduce pollution in the area.

During March and April of 2011, the following conclusions were reached: a) *T. catappa* trees on the central Pacific coast of Costa Rica produced a large number of seeds where natural food sources may have been scarce; b) there was evidence of resource partitioning of the seed resource between squirrels and macaws; and c) *T. catappa* trees were a very important component of scarlet macaw diets and the
resource size allowed squirrels and macaws to persist on the same resource.

ACKNOWLEDGMENTS

Thanks goes to the Associated Colleges of the Midwest, Costa Rica program staff for coordinating this study abroad program. Kathleen Shea provided important feedback on this project. Also, thanks to my (JIH) host families during my stay in Costa Rica. Finally, thanks to Punta Leona resort, especially Arnulfo Villalobos Arias for his assistance in the field. I would also like to thank The Parrot Society UK for funding different aspects of the project.

RESUMEN

Producción de semillas y depredación del almendro de playa (Terminalia catappa, Combretaceae) por lapas rojas (Ara macao) y ardillas chizas (Sciurus variegatoïdes). El conocimiento de los impactos ecológicos del almendro de playa exótico (Terminalia catappa) en el Pacífico Central de Costa Rica son poco conocidos, pero los estudios han encontrado que esta especie es una fuente de alimento potencialmente importante para la lapa roja (Ara macao), en peligro de extinción. En este estudio, se midieron la fenología reproductiva y la depredación de semillas por las lapas rojas (Sciurus variegatoïdes) y las ardillas chizas durante marzo y abril 2011 en las playas de la costa Pacífico Central de Costa Rica. Los niveles de productividad y depredación de semillas se cuantificaron semanales para 111 almendros de playa, para evaluar la importancia del almendro de playa como fuente de alimento para la lapa roja y el grado de repartición de recursos entre los depredadores de semillas. La producción de semillas de los árboles fue alta (cerca de 194272 semillas) y aproximadamente el 67% de las semillas fueron comidas por los depredadores, las lapas rojas consumen un estudiante de 49% de las semillas, mientras que las ardillas consumen el 18%. Adicionalmente, se encontró evidencia de la repartición de recursos entre las lapas rojas y las ardillas. Las lapas rojas prefieren alimentarse en el lado norte del árbol de la copa, mientras que las ardillas prefieren las regiones del sur y el interior de la copa. Ambas especies comieron la mayoría de las semillas en la parte del árbol con lado al mar. A pesar de la situación de este árbol como una especie exótica, la producción de semillas parece ser un recurso importante para la recuperación de la población de lapas rojas. El recurso que produce este árbol debe tomarse en cuenta para continuar con los esfuerzos de reforestación en Costa Rica.

Palabras clave: Ara macao, almendro de playa, Costa Rica, fenología, lapa roja, depredación de semillas, Sciurus variegatoïdes, Terminalia catappa, ardilla chiza.

REFERENCES


Species diversity and activity of insectivorous bats in three habitats in La Virgen de Sarapiquí, Costa Rica

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Abstract: Pineapple farms make up 45 000 ha of Costa Rican landscape and are the second most exported crop. This is economically beneficial for the Costa Ricans, but greatly affects the natural flora and fauna because it is such a low growing crop. This study examined the differences in insectivorous bat species diversity and activity in the habitat gradient between the forest in Tirimbina Biological Reserve in La Virgen de Sarapiquí, Heredia, Costa Rica and the nearby pineapple farm called Finca Corsicana. Over a four week period in March and April 2013, ultrasonic recorders were placed at different sites to pick up the bats’ calls. Then the recordings were analyzed to identify the species. There were four families present and 19 different species. There was a significant decrease in the number of bat passes (the number of times a bat passes the recorder) in the pineapple farm (\(\bar{x}=22.6\)), in comparison to the border (\(\bar{x}=39.9\)), and the forest (\(\bar{x}=44.2\)) (p=0.0028). Agricultural environments affected and lowered bat presence. Also, a greater mean number of bats recorded between 1900-1930hrs compared to 1730-1800hrs, coincided with the setting of the sun and beginning of bat activity. More research is need throughout the night and the year to establish clearer patterns of bat use and activity in different habitats.

Key words: bat activity, Costa Rica, habitat gradient, insectivorous bats, tropical rain forest, ultrasonic recorders.

Conservation efforts are being made all over the world to help protect the environment from human destruction. In order for these conservation efforts to be implemented, there must be an understanding of: a) how changes to the landscape affect the flora and the fauna, and b) how to correct the problem or at least create a balance between the needs of humans and the natural life. This study focuses on how the gradation in diversity between a biological reserve (protected forest), the border of the forest, and a pineapple farm affect bat species diversity and activity.

Pineapple is the second most exported crop in Costa Rica. Of the pineapples consumed in the US and Europe, 85% and 71% are from Costa Rica, respectively (Ingwersen, 2012). This makes pineapple economically important to Costa Rica. As of 2011, there were 45 000 ha of land cultivated in pineapple in Costa Rica. However, because it is a low-growing plant it drastically changes the landscape and thus the habitat of many species. Can bat species adapt to these new environments?

Miller, Arnett, and Lacki (2003) examined 56 bat studies on habitat management of forest-roosting bats and concluded that without roosting areas, bats will leave. Thus conservation efforts should be made to maintain roosting areas. It is highly beneficial to farmers to keep bats in an area because insectivorous bats greatly decrease pests and frugivorous and nectivorous bats help with pollination. It has been estimated that a colony of big brown bats (\textit{Eptesicus fuscus}) can eat 1.3 million insects a year, or each bat can eat 4 to 8 g per night. In monetary terms this is around $74/acre that farmers in south-central Texas, USA save in pesticides (Boyles, Cryan, McCracken, & Kunz, 2011).
Of 1,116 species of the bats in the world, 113 are found in Costa Rica and about 60 are known in the rural town of La Virgen de Sarapiquí, where this study was conducted (Laval & Rodríguez-Herrera, 2002). Around 75% of all bat species are insectivores, the rest eat fruit, nectar, fish, birds, or blood (Bat Conservation International, Inc., 2011). Even though insectivorous bats make up the majority of bat species, there are fewer studies on insectivorous bats in comparison to other types because insectivorous bats usually fly higher than mist nets, which are typically used in bat studies. The solution to this problem is recording devices.

Within the past 15 years there has been an increase in sonic recorder use to identify bat species difficult to catch in nets. Initially, research groups worked to identify all sounds made by each bat species by capturing them and recording calls of each species in open rooms. With species recordings, they were able to identify bats in the field with recording equipment (O’Farrell & Miller, 1999; Rydell, Arita, Santos, & Granados, 2002; Jung, Kalko, & von Helversen, 2007). Evidence of the usefulness of recording calls was shown in a study (Sampaio, Kalko, Bernard, Rodríguez-Herrera, & Handley, 2003) that focused on bat species adaptability to new surroundings in the Amazon Basin in Brazil with mist net captures at ground and canopy levels. However, sonic recordings detected 15 bat species not caught during the 2.5yrs of the study. MacSwiney, Clarke, and Racey (2008) specifically tested how effective the recorders are in the identification of bat species and found that mist nets missed 30% of bat fauna and aerial insectivores (insectivorous bats that do not land to catch insects) were not caught at all; they were only identified by recorders.

The use of recording devices is possible because the majority of bat species use echolocation to navigate and find food during the night (Rodríguez-Herrera & Montero, 2009). The bats send out the call and use the echo that bounces back to form an image of their surroundings, called an eco-image. This eco-image includes information about the form and the texture of an object. The frequency of these calls is higher than the human range, which stops at 20kHz. One bat can call as many as 400,000 times per night. Bats use two types of calls, the frequency-modulated (fm), which is in a wide frequency band for a short period of time, and the quasi-constant frequency (qcf), which is in a narrow frequency band for a longer period of time (Schmitzler & Kalko, 1998). Each species’ fm and qcf calls are different, which is how recording devices can be used to identify different bat species.

The main objectives of this study were to determine species richness in a gradient of disturbance in La Virgen de Sarapiquí. Through the use of sonic recorders, this study aimed to identify bat species in order to determine their activity and diversity within: a) the forest at Tirimbina Biological Reserve, b) the edge of this forest, and c) the edge of the pineapple farm, Finca Corsicana.

This will help build a better understanding of the interaction between bats and humans based on the effects of agriculture and create a better understanding of how best to protect bat habitats.

MATERIALS AND METHODS

Study area: This study took place in La Virgen de Sarapiquí, Heredia, in northcentral Costa Rica (10°24’41” - 10°27’05” N, and 84°06’52” - 84°07’43” W) during March and April 2013. La Virgen is a small town that runs along the Sarapiquí River. About 7km to the north of the center of La Virgen is Tirimbina Biological Reserve and about 2km further North, along Route 126, is the Finca Corsicana, a pineapple farm. The majority of the study was done in Tirimbina Biological Reserve, which is 345ha of protected forest (Tirimbina Biological Reserve, 2010) (Table 1).

Habitat sites: There were three main habitat sites: 1) the forest, 2) forest border, and the 3) pineapple farm. The forest was part of Tirimbina Biological Reserve, which is 98.55%
The 12 recorder-collection sites and their GPS coordinates

<table>
<thead>
<tr>
<th>Site</th>
<th>GPS Coordinates</th>
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<tbody>
<tr>
<td>A1 Second Bridge</td>
<td>10°24’51” N, 84°07’07” W</td>
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<tr>
<td>A2 Open Building</td>
<td>10°24’58” N, 84°07’23” W</td>
</tr>
<tr>
<td>A3 House 1</td>
<td>10°25’37” N, 84°07’43” W</td>
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<tr>
<td>B1 Corteza Trail</td>
<td>10°24’44” N, 84°07’03” W</td>
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<tr>
<td>B2 Cacao Farm</td>
<td>10°24’41” N, 84°07’18” W</td>
</tr>
<tr>
<td>B3 School</td>
<td>10°25’37” N, 84°07’43” W</td>
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<tr>
<td>C1 Hunter Trail</td>
<td>10°24’34” N, 84°06’52” W</td>
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<tr>
<td>C2 Road to Field Station</td>
<td>10°25’12” N, 84°07’17” W</td>
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<tr>
<td>C3 La Quinta</td>
<td>10°27’05” N, 84°07’17” W</td>
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<tr>
<td>D1 Botarrama Trail</td>
<td>10°25’04” N, 84°06’52” W</td>
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<tr>
<td>D2 Island</td>
<td>10°25’08” N, 84°07’07” W</td>
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<tr>
<td>D3 House 2</td>
<td>10°26’52” N, 84°07’38” W</td>
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Bat call collection: Each week one recorder (Song Meter SM2BAT+, Wildlife Acoustics, Concord, Massachusetts, USA) was placed in each of the three habitat sites and this process was repeated for 4wks. They were placed out on Monday or Tuesday and collected Saturday or the following Monday to ensure five whole nights of data collection. Table 2 illustrates the nights data was collected. Recorders ran from 1730hrs to 0530hrs. Each week, after retrieving the recorders, the data chips from the recorders were inserted into my computer (MacBook Pro) and the calls were transferred to an external hard drive. For each night there were eight files in wav format, each 1.5hrs in length. Then before moving the recorders to new recorder-collection sites the batteries were changed (only alkaline batteries will last the full length of time) and the data was deleted off the data chips in the recorders from the previous week to make space for new data.

Bat call identification: During daylight hours I analyzed the recordings using Raven Pro 1.5, (The Cornell Lab of Ornithology, Ithaca, NY, USA), a computer program designed to analyze sounds. I opened each file within this program, so that I was able to see 3min at a time. Passes were defined as the number of times a bat flew next to the recorder. Based on the definition by Estrada-Villegas, Meyer, & Kalko (2010), a pass ends when the time between two calls is three times longer than the time between the first two calls. I recorded the number of passes/species/ min in an excel spreadsheet.

To identify bat species, I compared the peak frequency, the length of the call, and the shape of the call to known calls (O’Farrell &
Miller, 1997; Fenton, Rydell, Vonhof, Eklof, & Lancaster, 1999; Kossol, Mora, Coro, & Vater, 1999; O’Farrell & Miller, 1999; O’Farrell, Miller, & Gannon, 1999; Siemers, Kalko, & Schnitzler, 2001; Mora, Macías, Vater, Coro, & Kossol, 2004; Jung et al., 2007; MacSwiney et al., 2008; Pio, Clarke, MacKie, & Racey, 2010; Jung & Kalko, 2011). Peak frequency is the frequency at the maximum power of the call. These researchers have captured different species and recorded their calls in order to accurately identify the species. Many of the articles mentioned above contained pictures of the sounds that I compared to the images in Raven Pro 1.5.

Time constraints hindered the analysis of all 12h from each night. Instead I analyzed the calls only from 1730-1800hrs and 1900-1930hrs for each night.

Differences between means of the variable, “number of passes”, were estimated between: a) the three habitats and two time periods; b) bat families and two time periods; c) bat families and three habitats; and d) four weeks and three habitats, with parametric two-way ANOVA. Differences between means of the variable, “number of species”, were estimated between: a) three habitats and two time periods with parametric two-way ANOVA. Assumptions were tested prior to execution of parametric ANOVAS (Sokal & Rohlf, 1995). Tests were executed with Statgraphics statistical software (www.statgraphics.com).

**RESULTS**

In 94 30-min segments over 4wks, several trends appeared. There were fewer bat passes for the pineapple farm ($\bar{x}=22.6$) than for the forest ($\bar{x}=44.2$) or border ($\bar{x}=39.9$) ($F=5.69$, d.f.=2, 312, p=0.0028, Fig. 1). There was a significant increase in the mean number of bat passes between 1730-1800hrs ($\bar{x}=25.4$) and 1900-1930hrs ($\bar{x}=44.3$) ($F=11.71$, d.f.=1, 313, p=0.0001). This trend was consistent for all habitats ($F=0.65$, d.f.=2, 309, p=0.5241, Fig. 1).

**TABLE 2**

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<td>D1 Forest</td>
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There was also a significant difference in the mean number of species per habitat and 30-min period (F=6.98, d.f.=2, 88, p=0.0015, Fig. 2). At 1730-1800hrs, there were more species in the forest (3.69) and border (3.59) than during 1900-1930hrs (forest 3.0, border 3.47). The opposite occurred at the pineapple site where there were fewer species during 1730-1800hrs (2.18) than during 1900-1930hrs (4.06).

Four families of bats were found: Emballonuridae, Molossidae, Vespertilionidae, and only two passes of Moormopidae (removed for statistical testing). Of the four families, 19 different species were identified (Cormura brevirostris, Cynomops greenhalli, Cyttarops alecto, Diclidurus albus, Eptesicus brasiliensis, Eptesicus furinalis, Euderma maculatum, Eumops spp, Lasiusurus ega, Molossus currentum, Molossus molossus, Myotis albecens, Myotis nigricans, Peropteryx kapleri, Peropteryx macrotis, Pteronotus pumilus, Rhogeessa io, Saccopteryx bilineata, Saccopteryx leptura). There was a significant difference in mean number of bat passes per family with Emballonuridae having the least (x̄=24.0), then Vespertilionidae (x̄=34.3), and Molossidae with the greatest mean number of passes (x̄=45.9) (F=4.32, d.f.=2, 308, p=0.0141). When families were compared to the three habitats there was a significant difference (F=3.33, d.f.=4, 302, p=0.0109). The mean number of bat passes for Emballonuridae was greatest at the border sites (x̄=40.5) and the smallest in the forest (x̄=11.5, Fig. 3). Mean number of passes of Molossidae was greatest at the border as well (x̄=54.4) and smallest in the pineapple farm (x̄=16.4, Fig. 3). In contrast, the greatest mean number of passes of Vespertilionidae was in the forest (x̄=53.7) and the least in the border habitat (x̄=28.3).

There was no significant difference in mean number of passes at each habitat between weeks (F=1.45, d.f.=6, 303, p=0.939, Fig. 4). For all four weeks the mean number of passes

![Fig. 1](image1.png)  
**Fig. 1.** Mean number of passes recorded for each habitat for each 30-minute period. Vertical lines are ±1 Tukey HSD confidence intervals (95.0%). Two-way ANOVA with insignificant interaction (F=0.65; d.f=2, 304; p=0.52). March-April 2013, La Virgen de Sarapiquí, Costa Rica.

![Fig. 2](image2.png)  
**Fig. 2.** Mean number of species recorded per habitat for each 30-minute period. Vertical lines are ±1 Tukey HSD confidence intervals (95.0%). Two-way ANOVA showing significant interaction (F=6.98; d.f=2, 88; p=0.0015). March-April 2013, La Virgen de Sarapiquí, Costa Rica.

![Fig. 3](image3.png)  
**Fig. 3.** Mean number of passes recorded for each family in each habitat. Vertical lines are ±1 Tukey HSD confidence intervals (95.0%). Two-way ANOVA showing significant interaction (F=3.33; d.f=4, 302; p=0.011). March-April 2013, La Virgen de Sarapiquí, Costa Rica.
per habitat increased, decreased and, except for the pineapple farm, increased again in week D.

Between the two 30-min periods was an hour of time. This hour created a significant difference in the activity of the different families (F=6.61, d.f.=2, 305, p=0.0015, Fig. 5). There were fewer passes for the Emballonuridae (\(\bar{x}=11.57\)) and the Molossidae (\(\bar{x}=23.06\)) during 1730-1800hrs than between 1900-1930hrs (Emballonuridae \(\bar{x}=35.53\), Molossidae \(\bar{x}=64.40\)). Mean number of passes of Vespertilionidae changed little and unlike the other two families, more passes occurred during 1730-1800hrs (\(\bar{x}=36.14\)) than during 1900-1930hrs (\(\bar{x}=32.64\)).

**DISCUSSION**

The main objective of this study was to examine the effect of habitat gradation on bats and results showed a difference in bat activity with the greatest mean number of passes in the forest and decreasing to the smallest mean number of passes in the pineapple farm. No significant difference existed in mean number of species between habitats, but a significant difference existed in mean number of passes per family in each habitat.

Based on different calls, different families seemed better suited to different habitats. For example, Vespertilionidae use fm calls with a long frequency and short duration, making them suited for forest habitats, where the greatest number of passes occurred (Schnitzler & Kalko, 1998). Voigt and Holderied (2012) found Molossidaes better suited for open spaces partly because of their physical conformation (body size and wing length) and their calls are qcf, almost cf, which means they have a small change in frequency and a longer length. In their study of *M. currentium* and *M. sinaloe* in open and closed spaces, the *M. currentium* performed better in the dense forest-like areas than the *M. sinaloe*. In my study there were a number of *M. currentium* and no *M. sinaloe*, which may explain why there was a greater number of Molossidae found in the forest and the border than the pineapple farm. Alpízar, Víquez, Hong, Rodríguez-Herrera, and González-Mayo (2012) in Tirimbina, with bat species in dense and open spaces within the forest, found greater numbers of Emballonuridae in the forest than the open spaces, but overall there was more species richness in open spaces (Jaccard=0.308). I found more Emballonuridae in the border than the other two habitats. Emballonuridae use gcf calls, which have both a large change in frequency and a long length of time making them ideally suited for border spaces (Schnitzler & Kalko, 1998).
While there was no significant difference in number of passes per habitat between weeks, there were some interesting trends that can be explained by the individual recorder-collection sites. In week B, the mean number of passes in the forest and the border were almost the same (forest $\bar{x}=48.07$, border $\bar{x}=48.14$). During week B the forest recorder was placed low to the ground, which could have prevented the microphone from picking up all of the calls and the border site was in the cocoa plantation, which is along the Sarapiquí River, but the recorder was more in the plantation than on the edge of the forest. In week C the forest recorder was placed in a tree on the side of a slope that was above a small river, thus creating more of an open space than forest space, which could have lead to a decrease in passes ($\bar{x}=27.0$). The forest recorder in week D was the furthest from the trail than in the other weeks creating a denser environment which may be why there is the highest mean number of passes at this site ($\bar{x}=65.60$).

The last major trend was the increase of mean number of passes between 1900-1930hrs when compared to 1730-1800hrs ($p=0.0001$). Bats are nocturnal species and the sun sets around 1730hrs, so by 1900hrs there has been more time for the bats to wake up and head out to feed. Jung & Kalko (2010) noticed a decrease in bat activity after 2030hrs in their human populated site, but no change throughout the night in their forest site. More analysis would have to be done of the bat calls from my study in order to clearly notice a trend in bat activity throughout the night.

ACKNOWLEDGMENTS

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RESUMEN

Diversidad y actividad de especies de murciélagos insectívoros en tres hábitats de La Virgen, de Sarapiquí, Costa Rica. Hay 45 000ha de piñeras en Costa Rica. La piña es la segunda fruta de mayor exportación en el país, por lo cual es muy importante para la economía. Este cultivo beneficia al país en términos económicos; no obstante, tiene efectos negativos en la flora y la fauna de áreas naturales al ser una planta herbácea de baja altura. Esta investigación examinó la diversidad y actividad de los murciélagos insectívoros en el gradiente entre un bosque (Reserva Biológica Tirimbina) y una piñera (Finca Corsicana) en la Virgen, Sarapiquí, Costa Rica. Durante cuatro semanas, puse grabadoras en tres sitios (bosque, borde de bosque y piñera) para grabar las llamadas de los murciélagos. Después analicé las llamadas para identificar las especies. Encontré cuatro familias y 19 especies. Hubo una reducción en el número de pases de murciélagos (número de veces que un murciélago vuela al lado de la grabadora) en la piñera ($\bar{x}=22.6$), en comparación con el borde de bosque ($\bar{x}=39.9$) y el bosque ($\bar{x}=44.2$) ($p=0.0028$). El ambiente agrícola afectó y redujo la presencia de murciélagos. Familias diferentes parecen estar adaptadas a hábitats diferentes. Finalmente, un mayor número de murciélagos fueron registrados entre las 19:00 y 19:30 hrs, comparados con las 17:30-18:00 hrs, lo cual coincide con el atardecer y el inicio de la actividad nocturna de los murciélagos. Se necesita más investigación, tanto en horas nocturnas, como del resto del año, para entender mejor los patrones de actividad y el uso de diferentes hábitats.

Palabras clave: murciélagos insectívoros, actividad de murciélagos, grabadoras ultrasónicas, gradiente de hábitats, bosque tropical lluvioso, Costa Rica.

REFERENCES


